

nature

November 6, 1975

Do scientific journals need a code of practice?

THE Scientific Information Committee of the Royal Society has recently put forward a set of guidelines for the refereeing of papers for publication. The status of the guidelines is unclear in the context in which they have been presented (as part of the programme for a conference of editors), but it seems that for the present they are open for discussion, and that even after such discussion they will only represent an informal set of rules to which editors may or may not choose to conform. We believe that such a voluntary code of practice is unworkable, will cause more trouble than it is worth and should be quietly dropped.

The committee is right in one respect: there is a growing feeling that the processes by which scientists regulate each other's activities, be it through grant-giving, refereeing and editing, or even control of membership of learned societies, could with profit be made somewhat more open. There are still, we think, strong objections to a system in which all is revealed and the names of referees and advisers are made public. But there are obvious intermediate positions in which the supplicant at least knows what sort of treatment to expect and can thus choose his own strategy accordingly. Unfortunately the issuing of guidelines suggests a move to uniformity, whereas what is actually needed is a preservation of diversity to allow individual scientists in individual cases to make individual choices.

Furthermore, once a set of guidelines is published there will be a tendency for some aggrieved authors to look to the guidelines for redress, even if the journal has never claimed to conform to them.

In matters of dispute it is clear that the drafting of the document leaves much to be desired, as loopholes abound. Three of the guidelines with which we disagree strongly are:

- Every paper submitted to a journal for publication should be refereed.
- No paper should be rejected on the adverse report of a single referee.
- A definite procedure should be established for editorial decision within a stated period.

However worthy these statements are, they are unworkable—indeed undesirable—in particular instances; and it is often in these very instances

(admittedly rare) that the author is looking for a way to prolong the battle. What is one to do about the author who scatters his paper with unsubstantiated statements, demands that not one but two referees should see it and insists that the journal should decide within a stated period? Supporters of the guidelines say that this is where editorial discretion comes in and of course “every” doesn’t mean “every”. But this leaves us with an additional guideline—‘the editor may choose to ignore these guidelines’. From a straw poll it is clear that many editors by no means conform and have no intention of doing so. It will surely bring the guidelines into discredit if they are widely ignored.

An alternative response to calls for more openness should be considered. If journals were to state regularly what their policy was in handling manuscripts, and the extent to which editorial discretion entered, prospective authors would actually have a clearer impression of how they stood.

With this in mind we have compiled the following brief statement of our editorial procedures.

Nature aims to publish scientific reports which are suited to a wider readership than a specialised journal can provide; manuscripts concerned with any branch of science will be considered. The spectrum of papers published broadly represents that of papers submitted.

All papers are assessed by one of the three manuscript editors (two, a biologist and a physical scientist, based in London; one, a biologist, based in Washington). These three editors may consult with other editorial staff and will return roughly a quarter of all manuscripts at an early stage, chiefly on the basis that they do not have any obvious broad appeal. The remainder are sent to one referee (occasionally two) and the referee's recommendation is generally followed. On rare occasions a negative report is overruled when there seems to be a pressing need for a particular paper to be made widely and rapidly available. Somewhat more often a paper that has received only a lukewarm recommendation from a referee is declined for reasons of limited space. Specific comments from a referee are usually passed on to authors.

The objective is to make a decision in principle on papers within a month of receipt, although authors are often asked at that stage to revise or shorten manuscripts. The present acceptance rate for submissions to *Nature* is around 35%, and following acceptance we aim to publish papers within six weeks.

We propose to publish an updated version annually.





Victory celebrations in Hanoi: pace of scientific organisation quickens

Vietnamese journey

Any country aspiring to an industrial and technological competence must have an underlying strength in basic science. The North Vietnamese know this, and taking their characteristic long view, have been organising their war-depleted scientific cadres into a versatile research nucleus. Their effort proceeded all through the war at whatever pace was possible, but wholesale evacuation of educational and research institutes to the countryside prevented rapid progress. As American participation in the war diminished and then ceased altogether, the pace of Vietnamese scientific organisation quickened. In June 1975, a scant six weeks after the formal end of hostilities, there were already strong indications that the Vietnamese will soon have high competence in the basic physical and biological sciences, as well as in the related applied fields of engineering, medicine and agriculture.—Arthur W. Galston reports.

AS a representative of the Scientists' Institute for Public Information (SIPI) and carrying a gift of books, chemicals and equipment from the Society for Social Responsibility in Science, I had been invited for a return visit to North Vietnam in June 1975. My first visit, made in April 1971 together with Ethan Signer of MIT, had led to interviews with Prime Minister Pham Van Dong, the Ministers of Higher Education, Public Health, and Culture, and visits to numerous scientific institutions. Now in a Vietnam freshly at peace, I had been invited to lecture in my speciality, plant physiology and biochemistry, to instruct young scientists in techniques of plant cell and tissue culture, and to initiate a scientific aid programme from the United States (through SIPI) to the new Vietnam.

About four miles north-west of Hanoi, a large, modern science research centre is nearing completion. Financed

by the USSR and constructed under Soviet supervision with Vietnamese labour, the centre was originally planned and administered by the State Committee on Science and Technology, the leading scientific policy making group in Vietnam. Since the end of the war, and to insure rapid progress, the centre, under its newly appointed director, Nguyen an Hieu, has been placed directly under Prime Minister Pham Van Dong, to whom Hieu reports directly. At a private conference with these two men, I was struck by their close rapport, and by the Prime Minister's understanding of and direct concern with scientific matters. Almost unconsciously, I had to contrast this with the situation in the United States, where in spite of its vastly greater competence and importance, American science has had no science adviser close to the President since Richard Nixon dismantled the structure that had been

created and successively strengthened by his five predecessors.

Hieu is a 39-year-old theoretical physicist who received his training under the noted Professor Bogolyubov at Dubna, the huge research centre near Moscow run by a consortium of Warsaw Pact nations. In addition to his role as director of the centre, Hieu directs the Institute of Physics, one of the six operating units of the centre. Since the completion of his PhD training in 1961, Hieu has made substantial contributions to the field of elementary particle theory, and now, as a member of the governing council at Dubna, visits that establishment every year. Five of his students have also received their basic training at Dubna, and in recognition of this growing Vietnamese competence in physics, the Dubna consortium recently donated advanced research equipment worth, conservatively, half a million dollars to the centre. This equipment is now largely set up and functioning in a series of huts and basement rooms of the centre, but with the imminent completion of that structure, will move into more spacious, even elegant, quarters.

Aware of the potential practical importance of research in solid state physics, Hieu has recently shifted to that field, and the laboratory's organisation reflects that emphasis. Already, large germanium and bismuth-tellurium single crystals have been grown, their impurities removed in a diffusion separation furnace, and the resulting purer crystals thinly sectioned to furnish the elements of semiconductor diodes for amplifiers. Sprinkled around the laboratory is a mixture of advanced equipment from many nations in the Soviet bloc, as well as the latest in scientific publications from all over the world, efficiently and cheaply reproduced by the Chinese without royalty payments. I saw a large Soviet neutron generator in the activation analysis of minerals and proteins, several multichannel data analysers from East Germany, a computer of Hungarian design based on American integrated circuits, and an advanced spectroscopy laboratory with many components from France, Great Britain and West Germany. A large X-ray laboratory is involved in analysing the structure of crystals and a solar energy group is using semiconductors to translate light energy directly into usable quantities of electricity. These activities would be thoroughly familiar to western physicists, and it is clear that besides playing a role in the development of science in their own country, Vietnamese physicists are eager to enlarge their contacts with the rest of the world.

I could get no exact figures on the cost of the Science Research Centre but I judge its approximate area at

500,000 square feet of usable space. At even \$20 a square foot, a ridiculously low level by western standards, the building alone will be worth \$10 millions when completed. Together with its abundant scientific equipment, it should eventually be worth about \$25 million. Though not especially impressive by American standards, this is enough to make it one of the largest installations in Vietnam. In the North, it will probably be second only to the \$75 millions paper mill being donated and built by the Swedes under an official aid programme.

In addition to the Institute of Physics, the centre will house the Institute of Mathematics, founded in 1969 under Le Van Thiem, a German-trained PhD. The still embryonic institutes of biology, earth sciences and oceanography are as yet still without directors. A chemistry laboratory devoted to the study of catalysis will serve as a nucleus for the ultimate development of an Institute of Chemistry, for which the Vietnamese judge they do not yet have sufficient competent personnel. A computer centre will complete the presently contemplated facilities.

I was, in fact, the first visiting foreign scientist in what Hieu envisions as an expanding programme central to the intellectual life of the centre. My lectures on plant physiology and biochemistry were attended mainly by those biologists close to that area, but I was impressed with the fact that physicists attended when I spoke on effects of light on plants, and both animal and microbial biochemists attended when I talked on the biochemistry of plant growth hormones.

I instructed a small research group in some new biological techniques and found the young scientists well trained in basic concepts and eager to learn, though several years out of date. They were proud to be the occupants of the first three finished rooms in the entire centre. These had been completed much before schedule in connection with plans for my visit, and the Soviet engineer and architect came around during one working day to ask whether I found everything satisfactory. That struck me as meaningful *detente* in action.

Though the centre is the most competent of the research facilities in North Vietnam, it is certainly not the only one. Much work in strength of materials goes on at the Hanoi Polytechnic Institute, in vaccines at the National Institute of Hygiene and Epidemiology, in liver cancer at the Viet Duc Hospital, in natural plant products and pharmaceuticals at several institutes, and in agronomy and herbicides at the Agricultural University. By contrast, the University of Hanoi has piti-

fully few laboratories for modern research, despite its major educational role. The biologists there listened with obvious envy as the American programme of aid to the Science Research Centre was outlined. Couldn't we help them too, they wanted to know. But they understood that our present limited resources would make no impact at all if too widely disseminated. They felt somewhat better when I promised to seek specific aid for them from other American sources.

Though scientific groups were understandably eager to receive American aid, official or unofficial, there was general agreement that American scientists have a special responsibility to continue investigations on the yet largely unknown consequences of the massive military defoliation operations in the South. In the decade starting about 1971, we dropped from aeroplanes about one hundred million pounds of chemicals toxic to plants over an area of South Vietnam greater than that of the state of Connecticut. About a quarter of South Vietnam's dense upland forests, one-third of its coastal mangroves, and perhaps one-tenth of its agricultural lands, were sprayed in an attempt to deprive the guerillas of forest cover and food resources. In spite of several Department of Defense-sponsored studies by US scientists, the ultimate ecological effects of this massive operation are not yet known, but several immediate effects, especially in the mangroves, are known to be severe.

Perhaps more important is the danger to public health, for it is now known that one of the herbicides used, 2,4,5-T, contained significant quantities of an impurity called TCDD (2,3,7,8-tetrachlorodibenzodioxin). Very small quantities of this material, in the parts per thousand million range, can cause death, the malformation of developing embryos in laboratory rats and mice, and chromosomal aberrations in dividing plant and animal cells. The latter observation raises the possibility that

TCDD can also cause mutations and cancer. Dr Ton That Tang is suspicious that the recent marked upsurge in liver cancer all over Vietnam may be related to TCDD persistence in the soils of sprayed areas. If this chemical were slowly released into streams, it would find its way into the algae and fish of the ocean, and ultimately into the human diet. Furthermore, since the ocean currents carry waters from south to north, Tung feels that TCDD sprayed in the South could affect civilians in the North. All this evidence is circumstantial, but since TCDD was detected in fish collected in 1973, two years after the herbicide spraying programme had been terminated, and since its persistence in soil and water is not known with certainty, the possibility of contained danger to public health and ecosystems continues.

Can the US turn its back on this problem? Or does American responsibility for investigation continue? Whether or not the US ever decides to give official scientific aid to Vietnam, many feel that it has a moral obligation to follow through on this research, much as it did after dropping an atomic bomb on Hiroshima.

Ta Quang Buu, a dynamic, Cambridge-educated mathematician, has served as Minister of Higher Education in North Vietnam since 1965. When I first met him in Hanoi in 1971, he impressed me with his keen, driving intelligence and his energetic devotion to the ideal of the best possible educational system for his country's youth, even in the midst of a debilitating war. But like many officials in the new Vietnam, Ta Quang Buu is finding the problems of peace more formidable than the problems of war.

Consider some of the difficulties facing him. One quarter of North Vietnam's population of almost 25 million is now at school. The figures for the South are not yet precisely known, but they are assumed to be rather similar. For the past 20 years, North and South have gone their separate educational



Plant physiologists at a Galston lecture

ways; their aspirations, philosophies of education and methodologies have been as different as communist and capitalist orientations can make them. Can two such systems possibly be unified? Should they be? If so, should change be gradual or sudden? What good points in the southern educational system can be adapted to the North? How should one handle the problems of adult education, given the imminent demobilisation of hundreds of thousands of soldiers? Although the end of the war came with dramatic suddenness, Buu's deputies have already travelled to the South for liaison with their counterparts. As staggering as the problems seem to be, Buu and his colleagues are tackling them with the same stubborn determination that characterised Vietnam's long military struggle.

In considering how best to mobilise and unify the educational and scientific resources of the North and South, Buu has analysed the situation in the South. Before 1973, he told me, university education in the South was completely dominated by the French model. The outstanding characteristics of this model are, first, an emphasis on general education, with only scant technical or vocational training being offered; second, the complete autonomy of the various faculties in devising standards, curricula and entrance requirements; third, no selective examinations at entrance, but rather massive elimination of students along the way (recently 60% have been eliminated after the first year and between 75 and 90% have never graduated); fourth, no assurance of a job after graduation and thus no limitation on the number of students choosing an option.

This unplanned system has resulted in an asymmetric and, according to Buu, chaotic expansion of the student population in southern universities. They now number more than 120 000, and of these, fully 50,000 are studying literature and law, preparatory to a legal career. "What can we do with so many lawyers?", exclaims Buu. "Ways will have to be found to alter the course of their studies, to divert them to other occupations". In similar fashion, the many students in training for financial careers will be retrained "to make good clerks and civil servants for the new system", and some of the many potential secondary school teachers will be encouraged to seek other occupations.

By contrast, the North Vietnamese system emphasises educational quotas in the various specialities as part of the planning for the filling of anticipated job vacancies. It also includes stiff, competitive entrance examinations, minimum dropouts along the way, and limited autonomy of the individual faculties under a more centralised and highly codified system of national edu-

cation. Following this system, which Buu believes resembles the American more than the French, the North Vietnamese have admitted only 55,000 students to higher educational institutions. This represents a decline from the 70,000 students enrolled in 1973, caused by the raising of educational standards. Obviously, to integrate the northern and southern educational systems, some compromises must be made in standards for admission and dropout projections, as well as in educational objectives and practices.

Paradoxically, American educational innovations introduced into the South about 1973 have shaped the system more toward the northern model. For example, the introduction of two-year community colleges at Da Nang, Nha Trang, Tu Duc and several other locations accords well with plans in the North to reserve the established universities for the final year of more specialised training. Also, under American guidance, polytechnic institutes were established for the training of engineers and other specialists. This too fits in well with the North Vietnamese approach. Hanoi Polytechnic Institute, built during the war, with Russian aid, would seem familiar in basic organisation to a student at MIT or Caltech. With typical Vietnamese eclecticism, Buu proposes to combine the best aspects of these various systems into a new, indigenous approach. "Bilateral accommodation" and "smooth interpenetration" were some of the phrases he used to describe how the process might work.

Even before the final formula has been devised, educational institutions closed by the war have been reopened. For example, Hue University was back in operation by April 14, and the Medical and Pharmacy faculties in Saigon reopened in June, barely a month after the end of the war. Though a few teachers fled with the departing South Vietnamese officials, almost all, according to Buu, are fitting in well with the new system, and are actively seeking guidance in making the accommodation. This is especially important since the student body, previously limited to the privileged classes, will now include many peasants and workers, for whom new educational techniques will have to be introduced.

Buu believes that equality of educational opportunity has already been widely implemented in the North, through improvement of rural secondary schools, through extensive adult education programmes, and by giving preference in university admissions to those with some work or military service experience. This year, approximately 35-40% of the 10,000 students to be admitted to universities in the North will have such work experience.

Since there will be about 80,000 applications in all, this will give a substantial competitive advantages to the more experienced applicants. Yet, Buu is all for flexibility, and definitely rejects the Chinese model of requiring work experience before admission to the university. Especially gifted or critically needed students should not, he feels, be held to that requirement.

There are as yet no postgraduate training centres in Vietnam for work leading to the PhD or equivalent degree. During the war, it was the practice to send promising students abroad, especially to the Socialist countries of eastern Europe, for such training. In 1969, there were about 12,000 such students abroad; the number is now down to about 7,000 and Buu expects it to go even lower as centres for such training develop in the time when some promising candidates can be sent to western Europe Vietnam. But he also looks forward to and even the United States, especially for training in science and technology.

Can the United States play a role in the postwar educational reconstruction of Vietnam? Like Prime Minister Pham Van Dong, noted surgeon Ton That Tung and other officials I interviewed, Buu insists that the Vietnamese do not hate, and in fact in many ways admire Americans. Under proper conditions, he would be glad to receive American aid. Gifts of books and scientific equipment, lectures and seminars by visiting specialists and exchange by communication are possible and desirable immediately. Exchanges of teachers and students are not desirable or possible now, but may be within two or three years, after the reunification of North and South has been accomplished, and their major educational patterns have been firmly charted.

Since American science and technology has been so extensively used for destructive purposes all over Vietnam, Buu believes it would be uniquely appropriate for groups of scientists to lend their talents to the reconstruction efforts now under way. In the light of President Ford's and Henry Kissinger's stated opposition to any such official aid, assistance would have to be unofficial and "people to people". The joint project of the Scientists' Institute for Public Information and the Society for Social Responsibility in Science was, he felt, an appropriate model. But he is neither doctrinaire nor proud in this matter. He will accept any appropriate aid given in good faith, confident that the future will bring a normalisation of relations between his country and the United States. "Then", he says, his eyes lighting up, "the vast wealth and power of the United States can really be focused on the job of rebuilding our underdeveloped and battered country".

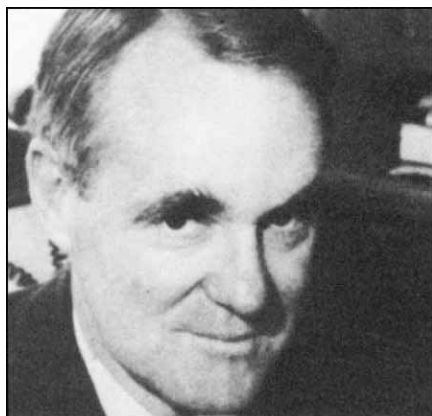
J W. CORNFORTH, who shares this year's Nobel Prize in Chemistry with V. Prelog, is now taking up, at the age of 58, a new full-time appointment as Royal Society Research Professor in the Department of Applied Science in the University of Sussex. He began his distinguished career in organic chemical research in Australia: he graduated at the University of Sydney and was awarded the University Medal in Organic Chemistry. This was followed by an 1851 Exhibition Overseas Scholarship to the UK, which he held from 1939 to 1942 in the Dyson Perrins Laboratory at Oxford with Robert Robinson, and he has remained in the UK since.

His early work was on Australian plant materials (with J. C. Earl) and he moved in Robinson's laboratory naturally. Inevitably in that environment he was attracted to the problem of the laboratory synthesis of cholesterol, and when the Second World War extended his stay in the UK, he was drawn into the maelstrom of the penicillin problem. In 1946 he joined the staff of the Medical Research Council at the National Institute for Medical Research and a career of individual work began to take shape; a summary is almost impossible to make: it would be a task of selecting highlights in a dazzling array of subjects. Enquiry into natural products, laboratory synthetic methods, fundamental reactions of biosynthesis, isotope techniques, reactions applicable to manufacture of medicinal products—all were grist to his mill; one has only to start discussion of a structural or synthetic problem with him to provoke a flow of 'graphite and cellulose' solutions. The only drawback to consultation is his complete deafness, which makes the problem of getting a word in edgeways and saying "yes, but" or

"I thought of that" a difficult one. The handicap of deafness he has overcome magnificently, particularly with the aid of his wife, Rita, who is herself an organic chemist of great skill.

Cornforth's relation with Robert Robinson is best put in his own words.

Cornforth's prize



Opening the Robert Robinson Lecture he gave to the Chemical Society in 1972 he said: "Our friendship . . . for more than 30 years has been a continuous sequence of differences of opinion". Reorientation of Cornforth's work, after the publication of some papers on miscellaneous subjects from the National Institute for Medical Research, came about in two ways in 1950. First, as a consequence of the Medical Research Council taking the lead in this country in the search for a source from which cortisone could be manufactured, and its involvement with the National Research Development Corporation, the workers at the National Institute were mobilised. Hecogenin, the steroid sapogenin from

sisal (*Agave sisalana*) was found to be such a raw material, which, on the basis of fundamental chemical work at Mill Hill and development in collaboration with the staff of Glaxo Laboratories became of considerable commercial importance.

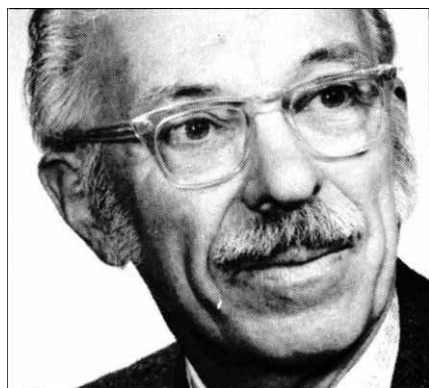
The second direction of development of Cornforth's work came in the early 1950s in association with George Popják, then in the Biochemistry Division at Mill Hill and later at the Medical Research Council's Radiopathology Research Unit. This work, which led, finally, to the results of fundamental theoretical significance for enzymology that have earned the Nobel Award, began with the examination of the problem of the route of biosynthesis of squalene from mevalonic acid. Development of new techniques for using isotopically labelled compounds, with such incidental ingenuities as the characterisation and orientation of the three hydrogen atoms of a methyl group by labelling with protium, tritium and deuterium followed, along with the extension to the study of enzyme stereochemistry on a wider basis.

In 1962, Cornforth and Popják became joint directors of the Shell Research Milstead Laboratory of Chemical Enzymology at Sittingbourne, an establishment that in the best tradition of schoolboy humour was dubbed at its inauguration the "Pop-Corn Laboratory". Unhappily Popják broke the link when he migrated to the USA. No doubt Cornforth's new laboratory in the University of Sussex will acquire some title of affection appropriate to the unique individuality of its presiding genius and certainly occupy an important place among the world's centres of scientific research.

R. K. Callow

Kennedy's good deed

THOMAS H. JUKES



ON September 8, the US Senate passed, by 61 votes to 29, a bill (S-963) introduced by Senator Kennedy to ban the use of diethylstilboestrol (DES) in growing beef cattle. The same bill would authorise the use of DES as a contraceptive after sexual intercourse only in rape, incest or "a comparable medical emergency" (the 'morning-after pill').

The bill carefully provides that the ban for beef must first be approved by the National Cancer Institute. This will be easy. Scientists of the National Cancer Institute have already testified before congressional hearings that one molecule of DES may cause cancer. Quite a lot of cancer cases were thus implied in a world population of 3×10^9 which is presumably exposed to molecular levels of DES in the air and water. One morning-after pill contains

7×10^{18} molecules.

Diethylstilboestrol is a known human carcinogen. Even worse, it is synthetic and man-made; a self-inflicted wound. Herbst, who discovered its carcinogenicity in human subjects, has calculated its cancer-causing potency: less than 4 cases per 1,000 in the female progeny of pregnant women receiving 1.5 mg (3×10^{18} molecules) of DES per day. He also notes that "there has been no documented correlation of maternal DES therapy with the development of cancer in males". Incidentally, DES is widely used as an anti-cancer drug in prostatic cases.

The intake of DES per capita from beef may be calculated from data for the residues found in implanted cattle, and the consumption of beef liver, 0.7 kg a year (0.12 parts per 10^9), plus beef muscle meats, 52 kg a year (0.012

parts per 10^9), in the USA. The intake corresponds to a daily dose of 1.9 ng, which is about 1.3×10^{-6} of the 1.5 mg used in Herbst's calculations. On a linear basis, the cancer risk to female offspring is, therefore, less than 5 cases per 10^9 , corresponding to less than one case per 133 years in the 1.5 million females born annually in the USA. How much less than one case?

Probably quite a bit, but actually unmeasurable in view of the fact that other oestrogens predominate over this level the efficiency of conversion of feed into DES. Nubile women secrete oestrogenic potency equivalent to 300,000 ng

of DES daily, and wheat germ contains oestrogens, measured by ingestion, equivalent to about 400 ng of DES per gram. Also, DES in beef liver is said to be present as glucuronide, which is inactive. The organic food crowd will applaud Senator Kennedy. More important, they will vote for him. Consumers will pick up the tab for the extra cost of beef production occasioned by the ban, which will decrease the efficiency of conversion of feed into lean meat by about 10%. Food prices are increasing so rapidly that this extra cost of up to \$900 million will be lost in the spiral.

Of course the money might have been used for cancer prevention and treatment . . . But who wants to take a chance on getting cancer, even one case in 133 years in 200 million people of a form of cancer that responds to treatment?

Banning DES will call for about 7 million tons of corn to maintain the US beef production at its present level. Senator Kennedy has professed sympathy for the plight of starving people in Bangladesh. Seven million tons is a lot of corn. But sympathy has to make sense. These people don't vote in Massachusetts!

international news

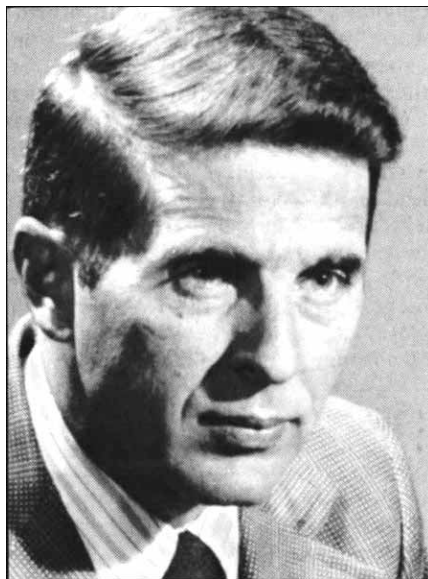
FOR years, spokesmen for the nuclear industry and the federal government have been assuring the American public that the risks associated with nuclear power plants are vanishingly small. The nuclear industry has a safety record unblemished by any accident which has released radioactivity into the environment, the argument goes, and nuclear power plants are bristling with so many safety devices that the chance of something going calamitously wrong is close to negligible.

But, as five years of raging debate have amply demonstrated, those bland assurances have not convinced everybody. So, in August 1972, the Atomic Energy Commission decided—though with some doubts about the feasibility of the project—to launch a mammoth computer analysis of the probability that a nuclear reactor could go haywire and endanger the lives of people nearby. The final results of the analysis, which were made public last week, support the official assurances. Nevertheless, they are unlikely to still the nuclear debate.

In general terms, the analysis suggests that there is a finite chance of a catastrophic nuclear accident, but the risk is minuscule when compared with other hazards such as fires, explosions, earthquakes, tornadoes or hurricanes. As William A. Anders, Chairman of the Nuclear Regulatory Commission (NRC)—which assumed responsibility for the project when the Atomic Energy Commission was dismantled earlier this year—suggested last week, the analysis “reinforces the commission's belief that a nuclear power plant designed, constructed and operated in accordance with NRC's comprehensive regulatory requirements provides protection to public

Computing the chances of a nuclear accident

by Colin Norman, Washington



William Anders: reassuring

health and safety and the environment”.

Carried out under the direction of Professor Norman Rasmussen, a nuclear engineer at MIT, the study involved a technique, widely used in the defence and aerospace industries, known as “event tree analysis”. In short, the technique attempts to predict the chances of failure of various components in a reactor and to assess the effects in terms of release of radioactivity, loss of lives and damage to property. The Rasmussen study explored thousands of potential sequ-

ences of failures in reaching its conclusions.

The final results, contained in a massive 12-volume report, conform closely to preliminary estimates published by Rasmussen in a draft report last year. Although the draft report attracted more than 1,800 pages of comment from various individuals and groups—much of it highly critical—the most significant change required in the analysis, according to Rasmussen, was a large increase in the estimate of the number of cases of cancer resulting from a massive release of radioactivity.

The study concentrated on the possibility of occurrence of a string of mishaps which would cause the core of a reactor to melt. Surprisingly, the analysis suggests that the chances of a core melt are about 1 in 20,000 per reactor year, which is rather higher than many previously quoted estimates (which have generally been put at about 1 in 10^6). But it suggests that the consequences of such an accident may not be catastrophic. “Contrary to commonly held belief that . . . core melting would surely result in severe accidents with large consequences”, the report states, “the magnitude of the potential consequences of a core melt accident were found to have a wide range of values. The probability is high that the consequences would be modest”.

The analysis suggests, in brief, that if a core melts, the pressure vessel which encloses it would not necessarily rupture and release large quantities of radioactivity into the atmosphere. The core would, however, eventually melt its way into the ground, but the study suggests that most of the radioactivity would be trapped in the soil. The most likely consequence of a core melt, the study predicts, would be less than one

death and less than \$1 million worth of damage to property outside the plant itself.

The worst possible case would involve a series of accidents leading to a core melt, followed by a massive explosion which ruptures the pressure vessel and releases large amounts of radioactive gases into the atmosphere, a steady wind blowing toward a populous area, and rain occurring at a critical time so that most of the radioactivity would be dumped on the population centre. The possibility of such a calamity would be about 1 in 10^8 per reactor year, the analysis suggests, but it would result in about 3,300 deaths and cause about \$14,000 million worth of damage to property.

The results of the analysis are stated in many ingenious ways in the report, the most widely quoted of which is sure to be the statement that a person living near a reactor stands a 2,000 times greater chance of being killed by lightning than of being killed by a nuclear accident.

Rasmussen said last week that he has high confidence in the accuracy of the predictions, and he pointed out that the methods used in the analysis have been endorsed by many groups. He readily acknowledged, however, that the study has its limitations. It does not deal with the possibility of sabotage, for example, but Rasmussen said at a press conference that it would be difficult to cause a very severe catastrophe by sabotage because of the existence of safety devices to prevent large releases of radioactivity following a core melt.

The analysis, moreover, is limited exclusively to an accident at an operating reactor, and does not include an assessment of the risks associated with waste disposal, the potential theft of atomic bomb ingredients by terrorist organisations, or other frequently cited hazards of nuclear power. The focus of the anti-nuclear power movement has recently shifted to those areas.

Needless to say, Rasmussen's conclusions have not met with widespread endorsement from groups which have been critical of the nuclear power programme in the past. Dr Arthur Tampin, a scientist on the staff of the Natural Resources Defense Council, last week suggested that the report has little application to the real world. "Those numbers are fine for an academic discussion in nuclear engineering departments", he said, but "the exercise has no reality". In particular, he questioned the study's predictions about what would happen after a core melt, suggesting that the behaviour of a mass of molten fuel is entirely unknown. Another critic was even as unchivalrous as to suggest that the report was a typical computer pro-

FOR the first time since 1969, the number of students enrolled in postgraduate science and engineering courses in the United States increased last year. According to a survey carried out by the National Science Foundation (NSF), the increase amounted to nearly 6% and the total number climbed back to the level of 1967.

Although the upturn makes a welcome change for many universities, which have been financially hard pressed and which have had to impose a virtual freeze on faculty recruitment partly because of declining enrolments in the early 1970s, the renewed interest in postgraduate education is of uncertain durability and it is concentrated heavily in a few fields.

The sharpest increase occurred in the life sciences, where the number of postgraduate students jumped by 11.3%. Psychology and the social sciences also showed substantial increases—8.0 and 6.6% respectively—while in engineering and the physical sciences there was only a slight upward trend. Engineering enrolments increased by 3.3% and those in the physical sciences by 1.4%; enrolment for the mathematical sciences declined a little.

The increases are surprising since they came at a time of continuing decline in direct government support of postgraduate students. The number of postgraduates receiving support from the federal government has declined by some 42% since 1967, and the trend shows little sign of abating because it is declared federal policy to phase out most graduate fellowship programmes. In contrast, the number of postgraduates supporting themselves through their studies is increasing—numbers were up by 14% between 1973 and 1974.

One explanation for the renewed interest in postgraduate education is that many scientists and engineers

who received their first degrees in 1973 were faced with a tight job market, and elected to stay on at the universities to increase their chances of finding suitable employment. As far as enrolment in the life sciences is concerned, the sharp increase probably reflects the fact that there is at present a fashion in the United States for studying medicine—a year or so ago it was law—and many students who failed to get into medical schools turned to closely related fields in the life sciences.

Does the upswing in graduate enrolments indicate a return to the golden years of university expansion in the 1960s? Almost certainly not. The driving force for expansion a decade ago arose partly from large increases in federal support for science and technology and partly from the fact that the post-war baby boom was passing through the universities. The federal science budget is now virtually stagnant in terms of spending power, and demographic trends suggest that there will be little expansion of the college-age population for the next two decades.

One particularly interesting fact to emerge from the survey is that the number of women enrolled in postgraduate science and engineering courses has been increasing sharply during the past few years. Women represented 24% of all full-time graduate students last year, and their numbers had increased by 13% since 1973. They were concentrated chiefly in the life sciences and the social sciences. The survey also reflects the fact that government restrictions on the employment of foreign students is continuing to depress their numbers. There were 3% fewer foreign students enrolled in postgraduate science and engineering courses last year, and their representation in the total postgraduate population declined to 16%.

grammer's nightmare of "garbage in and garbage out".

Nevertheless, the study is the most ambitious attempt so far to place hard numbers on the official assertions that nuclear power is safe, and it will clearly form the basis of nuclear regulation in the United States for many years. Rasmussen warned, however, that the results of the analysis should not be extrapolated beyond the first 100 or so operating reactors in the United States. Later reactors, he suggested, will be even safer because of constant improvements in the safety systems.

As a footnote, it should be noted that the report could actually be seen as a boon for critics of nuclear power.

At present, insurance against nuclear accidents is limited by law to a total of \$560 million per accident. The law, known as the Price-Anderson Act, has been bitterly contested by nuclear critics, who argue that in the event of a nuclear accident, full compensation should be paid for health and property damage. If the dangers are really as remote as the Rasmussen analysis predicts, and the consequences likely to be small, then why should there be any limits to the liability? A statement published last week by the Friends of the Earth suggested that the Price-Anderson Act "points out the hypocrisy and inconsistency of the federal government's policy towards nuclear power". □

Brazil and biodegradable detergents

from Bruce Handler, Rio de Janeiro

THE Brazilian Congress is studying a proposal to ban the manufacture of non-biodegradable detergents in this country, which is slowly waking up to the problems of pollution. The bill, which orders detergent makers to produce only non-polluting products, has passed the House Constitution and Justice Committee. It still must win the approval of the entire House, the Senate and the President to become law, however. Observers in Brasilia say the chances are good, in view of other recent indications that Brazil is abandoning its one-time policy of development at any price in favour of one that shows more concern for protection of the environment.

The detergent bill came as a result of a recent invasion of foul-smelling white foam that drifted off the Tiete River in the south-eastern part of the country and literally covered the Sao Paulo suburb of Santana do Parnaiba. The tropical town looked as if it had been hit by a freak snowstorm. Environmental experts were unanimous in blaming excess amounts of non-biodegradable detergents that had been dumped into the river as the principal cause.

Concern over the Santana do Parnaiba incident was expressed as far away as Argentina, because the polluted Tiete empties into the Parana-Plate river basin. Because of Brazil's relative lack of concern about pollution until now, it may be prohibitively expensive to implement the detergent bill, even if it is passed quickly. Brazil at present imports all the benzene distillates needed to make biodegradable detergents, and because of a disastrous balance-of-payments situation caused by the high price of foreign crude oil, the government has ordered a drastic cutback on all but the most essential imports. The situation is even more pessimistic when one takes into account the fact that detergents are responsible for only a small percentage of water pollution in Brazil.

In August, President Ernesto Geisel signed a decree saying that all industries in Brazil must control pollution and giving the federal government the power summarily to close factories that foul the environment. This is quite a change from Brazil's position on pollution just a few years ago, when it told an international conference on the environment: "A country that has not yet reached satisfactory living standards cannot sidetrack its resources for pollution control."

The new decree does not mean, however, that this fast industrialising nation of 110 million people is going to become a paragon of environmental protection overnight. In fact, many pollution-conscious people here felt the immediate purpose of Geisel's decree was to stop state and local government from cracking down too hard and too fast on polluters, and thereby interfering with national development plans.

Cabinet ministers who suggested the anti-pollution measure to the president noted that although Brazil has an "acute" industrial pollution problem, the government must be careful "not to disorganise productive activities or cause social unrest." This seemed to be fancy wording for the problem of citizens in several Brazilian cities who have shown themselves to be at their wits' end because of pollution—and who would take to the streets in protest, if public demonstrations were not illegal under the military-controlled regime here.

A typical case occurred recently in the industrial town of Contagem outside Belo Horizonte, Brazil's third largest city. There, a cement factory whose smokestacks spew out over 100 tons of particles a day on local residents' houses had been promising for years to install pollution control equipment but never did. The people of Contagem complained that the factory was responsible not only for the white dust that covered everything in sight but also for widespread respiratory ailments.

Mayor Newton Cardoso finally ordered the factory closed. He said that "the human being must always be the focus of the government's attention." State police were sent out to make sure the plant's executives began shutting down production equipment, to comply with a deadline the mayor had set. In addition, the DOPS—the much-feared political police—went to the cement factory after hearing reports that townspeople were going to hold a demonstration there to celebrate Mayor Cardoso's decision.

Then the presidential decree came out. A judge rescinded the mayor's order, on the grounds that local authorities could no longer single-handedly shut down factories. The cement plant continues to operate, although its owners have promised to install anti-air pollution devices in 1976.

Paulo Nogueira Neto, who holds the recently created federal post of special secretary for the environment in Brazil, summed up the dilemma: "The important thing is not to pollute. But we also cannot ignore the economic side of the problem." He added that the new presidential decree "makes it clear that pollution control is obligatory and that there will be no

loosening up."

Brazil has made remarkable economic progress since a development-minded but politically repressive government took power in 1964. But it has paid a high price in ecological terms.

Pollution in Sao Paulo, the nation's largest city, has become so bad that a leading department store now stocks anti-pollution masks—in adults' and children's sizes. Sao Paulo's last mayor won applause from the populace when he temporarily closed a polluting cement factory there. But this led to a political fight with the governor, and the mayor lost his job. The factory is open again, and the particle fallout is as bad as ever.

Local authorities in the southern state of Rio Grande do Sul succeeded in closing down a foul-smelling cellulose factory that had been built by a big Norwegian pulp and paper manufacturer. But that factory is now in business again. The Norwegians sold their share of the undertaking to private Brazilian interests, and an influential retired Army general was installed as president of the company.

Federal Representative Jose Roberto Faria Lima, a supporter of the bill to ban non-biodegradable detergents, made this comment: "It's too bad the Tiete River had to become covered with foam before the need was shown for a law requiring the production of biodegradable detergents in this country. Anti-pollution plans and even the President's decree don't do any good. Simply saying: 'It's against the law to pollute' doesn't stop pollution". □

Coal standard

from Vera Rich

DONETSK, in the Ukrainian SSR, has been accepted as the standard coalfield by the Eighth International Congress of Stratigraphic and Carboniferous Geology, which met recently in Moscow. The phenomena of the Donetsk field will be used as a basis for a unified international scale to be used in prospecting and establishing the boundaries of coal fields. The scale, which was proposed by a team of geologists from Britain, France, the USA and the Soviet Union, is at present at the draft stage. Nevertheless, the acceptance of Donetsk as the standard seems unlikely to be changed by further discussions, since this field is unique in giving a clear and detailed picture of the history of the whole of the Carboniferous period (345–280 Myr BP). Although Donetsk was selected on the basis of its geology, this coalfield has a history which may serve as a promising augury for international cooperation. As its original name of Uzhovka indicates, it was first opened up (1869–1870) by a Welsh mining expert by the name of Hughes. □

AN idea first put forward by Zionist visionary Theodore Herzl 73 years ago, and then brought up again four decades later by an American soil conservationist, Walter Clay Lowdermilk, may soon, at long last, be implemented.

Herzl, Lowdermilk and others have advocated the construction of a canal (or tunnel) through which seawater would flow from the Mediterranean to the Dead Sea so that the difference in level of 400 m could be exploited for electricity generation.

Advocates came and went, but only recently, prompted by the energy crisis, did the government appoint a committee seriously to consider the possible implementation of such a scheme. The committee, headed by Bar-Ilan Professor Shlomo Eckstein, had before it a plan for building a 75-km tunnel which would bring Mediterranean water to a reservoir in the Judean Desert highlands overlooking the Dead Sea, from which it would be 'dropped' through turbines with an installed capacity of 300 MW.

The next stage, if the Eckstein committee's recommendations are accepted by the government, will be a 'pre-feasibility study', including test drillings at various places along the proposed route.

According to present estimates, the project will require 10 years to complete and cost more than \$200 million (at present prices). In operation, it will not only increase electricity supplies but also replenish the rapidly evaporating waters of the Dead Sea, from which large quantities of potash, bromine and other valuable minerals are extracted. Traditional sources of replenishment, particularly the inflow from the Jordan River, have gradually dried up as more and more sweet water is being syphoned off upstream by Israelis and Jordanians for irrigation purposes.

There is, however, a limit to the amount of seawater that can be poured into the Dead Sea if it is not to overflow. Thus the turbines can only be activated for some 2,700 hours a year. As conceived at present, the flow would be scheduled to coincide with periods of peak demands for electricity, when hydroelectric power would supply 10% of Israel's estimated electricity requirements (in 10 years' time). Should other power stations be put temporarily out of action, however, the turbines could be kept in operation for an extended period, after which they would have to be shut down for two or three years while the Dead Sea 'dried up'.

The substantial quantities of water flowing through the proposed tunnel, planners say, could also be used for

cooling purposes by nuclear power stations, making it possible to build them away from the crowded seashore.

● Israel does not yet have a nuclear power station on the coast—or anywhere else for that matter—but every 'nuclear development' in the country arouses interest, often for the wrong reasons. This was demonstrated once again a few weeks ago when a Dutch newspaper printed a photograph of a tower-like object and captioned it as follows: "A new nuclear energy installation is being completed near Tel Aviv. No details about this installation at the Weizmann Institute are known. However, the great powers are not only interested, but also worried about Israel's growing atomic strength."

Letter from Israel

from Nechemia Meyers

In point of fact, the object shown is the 19-storey Koffler Accelerator Tower, next year to house a 14-UD Pelletron accelerator.

Far from being a secret facility, the Pelletron will be open to scientists from around the world. Indeed the first large international group to visit the site—participants in the recent Third Tandem Accelerator Conference—were there at the same time the Dutch newspaper was making its mistake.

● While Israeli physicists concern themselves, for the most part, with basic research, Israelis in the biomedical field generally concentrate on the solution of day-to-day problems. Such is the case with Professor Bruno Lunenfeld, Director of the Institute of Endocrinology at the Sheba Medical Centre, near Tel Aviv. Professor Lunenfeld, for example, has developed a method that enables previously childless Orthodox Jewish women to have children without violating the tenets of family purity as laid down by traditional Judaism.

For an Orthodox woman, sexual intercourse is only permitted during a limited period each month. It can begin only seven days after the end of her menstrual period, until when she is regarded as 'unclean'. In ordinary circumstances, this does not prevent her from becoming pregnant, but if a woman's menstrual period extends beyond the usual five days, or her menstrual cycle is less than the usual 28 days, her days of greatest fertility may occur during the time when she is 'unclean' and therefore forbidden to her husband.

Professor Lunenfeld found that this problem can almost always be solved, once the religious woman's most fertile days are determined, by giving her an

anti-oestrogenic substance known as clomiphene citrate. As a result, ovulation is delayed long enough to occur when the woman is permitted by the laws of family purity to have intercourse. Ironically, this substance was originally developed as a means of birth control, but is now used primarily to induce ovulation in infertile women.

Incidentally, Moslem women have also benefited from the treatment. Subject to even more severe laws of purity than are their Jewish sisters, the anti-oestrogenic substance has saved many of them from childlessness, a particularly tragic fate in traditional Moslem society.

● In another part of the Sheba Medical Centre, Dr Dan de-Medina is using the techniques of biofeedback to treat veterans of the Yom Kippur War.

Dr de-Medina, whose professional career began with a cancer research team and later continued in the sphere of memory studies, first delved into biofeedback when on an extended visit to the USA in 1973. He arrived back in this country, fortuitously, shortly before the outbreak of the Yom Kippur War, in the wake of which hundreds of men suffered from post-traumatic neuroses. And in spite of the fact that biofeedback techniques had never previously been used in Israel for the treatment of such neuroses, Dr de-Medina was allowed to use them with some of the badly shaken war veterans.

He found, as did others dealing with this problem, that it was more common and more severe than it had been after previous wars, presumably because the fighting had lasted longer and because the outcome had been less clear-cut (the euphoria of a great victory apparently has a healing effect). The patients sent by the army to Dr de-Medina generally returned to relative normality more quickly than those treated by other techniques, some of which, unlike biofeedback, involve the possible creation of drug dependence.

The method used by Dr de-Medina (and overseas practitioners) is quite simple. It involves connecting up the patient to a 'Galvanic Skin Response Feedback' instrument, not unlike a lie detector, which registers anxiety on a scale or by the sounding of a siren. After a period of conditioning, usually involving some 20 sessions, the patient finds he is able by his own efforts to reduce the number registered on the scale or the pitch of the siren and, almost as a side effect, to lessen his sense of anxiety. Finally, he gains a conditioned reflex whereby anxiety is switched off, without the benefit of the instrument.

correspondence

Industry and Academics

SIR,—I would like to comment on your leading article "Why industry and academics go their separate ways" (September 25).

Whereas in the large industrial countries, in spite of the problems cited in the article, there is probably always enough dialogue between research and industry to produce good results, in small countries like Norway scientific effort has to be very carefully husbanded. They cannot afford the luxury of *laissez faire* in research and development, and somehow they have to overcome the pre-development, evaluation and identification gaps, even if they don't satisfactorily solve them.

I have been living for much of the past 12 years in Norway, working in, or in the closest vicinity of, an interesting semi-academic institution which represents one solution to the problem presented in your article. This institution has been in existence for 25 years now, is still flourishing, and is therefore perhaps worthy of mention. It consists of a contract organisation by the name of SINTEF, situated on the campus of the Norwegian Technical University. It has a staff of some 650 people, of whom 350 are university graduates or the equivalent. In that it works hand-in-glove with the university, it is instrumental in keeping the latter in close touch with industrial trends, and in that most of its income is derived from industrial projects, it is unable itself to stray too far from industrial needs.

SINTEF is a non-profit-distributing organisation, with a board consisting of academics and industrialists. Money from the National Research Council is obtained largely according to the project in hand, and often on a 50-50 basis with an industrial partner. Good ideas generated internally are backed internally until ripe enough for presentation to industry, at which point they are then, in the ideal situation, developed into a marketable product at industry's expense. Good ideas, or requirements, from industry are brought to SINTEF for fundamental development because the latter has by now built up a solid reputation for quality, timeliness and (believe it or not) budget consciousness. A fairly thorough presentation of SINTEF will be published shortly in the *European Journal of Engineering Education*.

I often ask myself why this tripartite symbiosis actually works, and I think there are at least three good reasons. First, the university is not isolated from industry as so many are in larger countries. Second, a substantial number of research-oriented people get a great kick out of seeing their ideas actually transformed into a product, ensuring an adequate supply of employees. And third, Norway actually benefits from being small in that industry knows what SINTEF is, and can very easily get in touch with the appropriate people.

If there is a fourth important reason, I would say it is that a country like Norway simply doesn't have the national resources to turn research and development into a holy cow. Apply your research or starve. Perhaps one of the salutary results of Britain's tragic situation today will be precisely this.

NORMAN SANDERS

Trondheim, Norway

EEC directives

SIR.—Eric Ashby (October 16) says that "all pollution except that from atomic weapons is a by-product of processes which benefit society".

Lord Ashby must have walked sometimes through a busy street and been assailed by a barrage of traffic noise from cars and heavy lorries (all necessary?) and breathed in their exhaust fumes. Perhaps he might have gone into a restaurant or cinema and filled his lungs with tobacco smoke. There is a vast amount of packaging of food in plastic and the like, much of it for the sake of advertising. Many people are engaged for eight hours or more per day with sheer monotonous routine work making products which have more to do with status than with benefit. Can Lord Ashby honestly believe, therefore, that much of what is manufactured in our society is beneficial?

Why is it a phony argument for the EEC to assert that a Scottish mill discharging into the Atlantic will unfairly compete with a mill on the Rhine which has to comply with stringent standards? Surely, if a uniform standard were not applied to all factories, then only those factories which could pollute the environment unabated would remain in existence while others under stringent control would go out of

business. After all, Lord Ashby did mention that he was in agreement with the EEC's long term objective concerning the purity of the sea.

Lord Ashby did not like the idea of regular monitoring of the beaches for pollutants, and his main objection was that it would cost £100 million a year (a guess by the Department of Environment). He supports his objection by mentioning a report made by the Medical Research Council in 1959 which stated that, in spite of the smelliness and ugliness of many beaches, they were not a hazard to health. Even if the conclusion of the report were absolutely correct, has a report published 16 years ago any relevance to what is happening today and could happen in the future as regards the pollution of the sea?

The gist of Eric Ashby's article seems to me to be: 'We're not going to let a lot of foreigners tell us what to do'. The 'foreigners' seem, however, to be much more willing to tackle pollution than do the powers that be in Britain.

PAUL C. FRENCH

Blommenholm, Norway



A hundred years ago

At last Friday's lecture by Dr. Carpenter, in connection with the St. Thomas Charterhouse School Teachers' Science Association, Dr. Lyon Playfair presided. In proposing a vote of thanks to Dr. Carpenter, Dr. Playfair referred to the subject of compulsory education, which is gradually becoming universal in this country, but which, he said, would be pure tyranny unless the education in our schools was increased and its quality raised. Quantity is all very good, but unless there is quality along with it, there is not much gained. "If it was to be said that children of thirteen or fourteen years of age were merely to receive the same education would be but tyranny. Therefore compulsory education involved higher education." Dr. Playfair expressed his gratification that the teachers composing the Association had banded themselves together in order to qualify themselves by attending such lectures as those of the Gilchrist fund and by other means, to undertake this higher education, which, we believe with Dr. Playfair, will be forced upon us even in elementary schools by the spread of compulsory education.

from *Nature*, 13, 35, Nov. 11, 1875.

news and views

Humoral factors in myasthenia gravis

from Vanda Lennon

IN 1905 Buzzard speculated that the symptoms of myasthenia gravis (MG) might be caused by an "autotoxic agent which has a special influence on the protoplasmic constituent of voluntary muscle" (*Brain*, **28**, 438). Today two distinct protein entities have been identified as potential candidates for the role of the "autotoxin" mediating neuromuscular blockade in MG: antibody to acetylcholine receptor (*News and Views, Nature*, **256**, 10; 1975) and thymopoietin (formerly called thymine), a polypeptide derived from the thymus (Goldstein and Schlesinger, *Lancet*, **ii**, 256; 1975).

Purification of nicotinic acetylcholine receptor protein (AChR) from the electric organs of *Electrophorus electricus* and *Torpedo californica* allowed the establishment of an animal model of MG—experimental autoimmune myasthenia gravis (EAMG) (Patrick and Lindstrom, *Science*, **180**, 871; 1973; Lennon *et al.*, *J. exp. Med.*, **141**, 1365; 1975; Tarrab-Hazdoui *et al.*, *Nature*, **256**, 128; 1975). EAMG is readily induced in mammals by immunisation with AChR and resembles spontaneously occurring human MG by every criterion examined so far. Clinically, muscle weakness, fatigability and improvement by anti-cholinesterase drugs occur. Electrophysiologically, a decrementing response of muscle to motor nerve stimulation occurs at low rates of stimulation ($2\text{--}10\text{ s}^{-1}$) and rapid repetitive stimulation induces post-activation facilitation followed by exhaustion—phenomena which occur in MG. The electrical defect is repaired by anti-cholinesterases and sensitivity to curare is pronounced (Seybold *et al.*, *Ann. N.Y. Acad. Sci.*, in the press). The amplitude of miniature end-plate potentials (mepp's) is reduced, but the number of transmitter quanta released by a nerve impulse and the stores of acetylcholine quanta immediately available for release by nerve impulse are normal (Lambert *et al.*, *Ann. N.Y. Acad. Sci.*, in the press).

Ultrastructurally, motor end-plates of rats with chronic EAMG are indistinguishable from those of patients with MG, the post-synaptic membrane being the primary target of the autoimmune reaction (Engel *et al.*, *J. Neuropath. exp. Neurol.*, in the press).

The critical link between EAMG and MG has been the finding of antibody to AChR in the serum in both conditions (Almon *et al.*, *Science*, **186**, 55; 1974; Aharonov *et al.*, *The Lancet*, **2**, 340; 1975; Lindstrom *et al.*, *Ann. N.Y. Acad. Sci.*, in the press). Using human muscle AChR as antigen, Lindstrom and coworkers have found that anti-receptor antibody is specific for MG, being present in 87% of 71 MG sera tested (and also in newborn infants of a myasthenic woman) and in none of 156 subjects with other neurological, autoimmune and endocrine diseases. Interestingly, this antibody, like that in EAMG, is not directed at the acetylcholine binding site of the receptor. *In vitro* studies have shown, however, that anti-AChR antibodies from animals with EAMG diminish acetylcholine sensitivity of muscle fibres, reduce the mepp amplitudes and decrease the ability of AChR *in situ* to bind α -bungarotoxin (Green *et al.*, *Proc. R. Soc. Lond.*, **189**, 57; 1975).

Thymopoietin is a polypeptide which has recently been purified from bovine thymus (Goldstein, *Nature*, **247**, 11; 1974) and synthesised (Schlesinger *et al.*, *Cell*, **5**, 367; 1975). This polypeptide was named for its unequivocal capacity to induce bone marrow cells *in vitro* to express differentiation antigens characteristic of thymocytes (Basch and Goldstein, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1474; 1974). Additionally thymopoietin is reported to have an effect *in vivo* on neuromuscular transmission (Goldstein, *Lancet*, **ii**, 119; 1968; Goldstein and Schlesinger, *ibid.*, **ii**, 256; 1975). When thymopoietin was given to mice intraperitoneally in nanogram amounts an apparent neuromuscular block (which

did not increase with increasing doses) was found between 18 h and 5 d later. This contrasts with the effects of cobra venom toxins which block AChR *in vivo*; in rats these toxins cause weakness, which is dose related, and electromyographic decrements typical of MG beginning within 10–60 min and reaching a maximum by 3–13 h (Satyamurti *et al.*, *Science*, **187**, 1975; Lennon *et al.*, *J. exp. Med.*, **141**, 1365; 1975). The characteristics of the decrement ascribed to thymopoietin have not been reported and are not evident in the short published record. Unfortunately, the defect was demonstrated at a stimulation rate of 50 s^{-1} , a rate at which artefacts are difficult to avoid; stimulation rates of $2\text{--}5\text{ s}^{-1}$ are optimal for demonstrating decrements in the diagnosis of MG (Slomic *et al.*, *Brain Res.*, **10**, No. 1; 1968; *New Developments in Electromyography and Clinical Neurophysiology*, Desmedt, J., ed., **1**, 241–304, Karger, Basel; 1973). Thus, the mechanism of action of this interesting polypeptide requires detailed electrophysiological analysis but its relevance to MG is far from clear.

What then is the role of the thymus in myasthenia gravis? Abnormalities of the thymus (hyperplasia and tumours) are often found in association with MG, and selected patients benefit from thymectomy. About 30% of patients with MG have antibodies, demonstrable serologically, which react with striations of skeletal muscle and with similar elements in the thymus, the "myoid" cells. Nicotinic AChR also has been identified in the mammalian thymus (Lindstrom *et al.*, *Ann. N.Y. Acad. Sci.*, in the press; Aharonov *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1456; 1975). Thymuses of patients with MG typically have germinal centres, which are the hallmark of antibody production and are not normally present in primary lymphoid organs. Thus it is not surprising that MG thymuses contain a large proportion of B lymphocytes (Abdou *et al.*, *New Engl. J.*

Med., **291**, 1271; 1974) and that in tissue culture MG thymocytes, in contrast to normal human thymocytes, synthesise considerable quantities of immunoglobulin (Smiley *et al.*, *Clin. exp. Immun.*, **4**, 387; 1969). It has yet to be ascertained whether this immunoglobulin is anti-AChR antibody, which would mean that in MG the thymus is a site of autoantibody production. It was the presence of germinal centres in MG thymuses that initially led Goldstein to propose that the primary lesion in MG was an autoimmune "thymitis" which led to the release of large amounts of thymopoietin. Goldstein and Whittingham (*Lancet*, **2**, 315; 1966) reported induction of histological signs of "autoimmune thymitis" in experimental animals by immunisation with saline extracts of muscle and thymus tissue in adjuvant. Those animals had no signs of weakness but did exhibit an electromyographic abnormality similar to that described in the subsequent thymopoietin studies. Small reductions in the amplitudes of mepp's were reported in rats with "thymitis" (Goldstein and Hofmann, *J. Neurol. Neurosurg. Psychiatr.*, **31**, 453; 1968). The reduction in amplitudes was much smaller than that observed in patients with MG or in

rats with EAMG. Blind studies and attention to details such as differences in input resistance would seem essential in assessing the significance of the small differences reported in rats with "thymitis". On the basis of observations in EAMG, rats with so little reduction in mepp amplitudes would not be expected to show decrementing electromyograms. Considerable controversy has surrounded the model of "experimental autoimmune thymitis", some authors claiming confirmation of Goldstein's findings (Kalden *et al.*, *Clin. exp. Immun.*, **5**, 319; 1969; Kawanami and Mori, *ibid.*, **12**, 447; 1972; Mrozek and Kaminska, in *Structure and Function of Normal and Diseased Muscle and Peripheral Nerve*, (edit. by Petruszewicz, I., and Jedrejowska, H., 145 Polish Medical Publishers, 1972), others failing to confirm the findings (Vetters *et al.*, *Lancet*, **2**, 28; 1969; Kaufman *et al.*, *J. Neurol. Neurosurg. Psychiatr.*, **32**, 281; 1969; Jones *et al.*, *J. Neurol. Neurosurg. Psychiatr.*, **34**, 399; 1971). It seems imperative that the effects of thymopoietin on neuromuscular transmission now be re-examined and documented in as convincing a manner as have been its T-lymphocyte differentiating properties. □

laboratories established that transplantable teratocarcinomas could also be obtained from many different strains of mice by injecting (male or female) embryos of up to 7 days development (but not older) into extrauterine sites such as the kidney capsule.

When embryoid bodies of either testicular or embryo-derived tumours are cultured *in vitro* they attach to the dish and cells proliferate out to form a multi-layered sheet within which a whole range of differentiated tissues gradually develop (Teresky *et al.*, *J. Cell Physiol.*, **84**, 319-332; 1974, and Gearhart and Mintz, *Cell*, **6**, 61-66; 1975, for recent examples of this process). Single embryonal cells can also be isolated from embryoid bodies and used to obtain a clonal population of homogeneous cells which remain undifferentiated over many generations, so long as they are prevented from forming dense aggregates. The majority of these cells retain a normal mouse karyotype and have several morphological, biochemical and cell surface properties (review by Artzt and Bennett, *Nature*, **256**, 545-547; 1975) in common with cells of the early mouse embryo.

Recently, G. R. Martin and M. Evans showed that if teratocarcinoma cells are allowed to form floating aggregates, the outer layer differentiates to form endoderm (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1441-1445; 1975). So far, sorting out or differential proliferation of two cell populations has not been rigidly excluded. It seems more likely, however, that the cells on the outside of the aggregate receive a signal triggering them to differentiate as endoderm, a process which also happens during the normal development of the 4-4.5 day ICM. (Rossant, *J. Embryol. exp. Morph.*, **33**, 991-1001; 1975). What kinds of signals are responsible for triggering such changes in cell commitment is a crucial question in developmental biology, and if teratocarcinoma cells are to be of any use in solving the problem it is important to show that they are equivalent to embryonic cells in their ability to participate in normal morphogenesis and differentiation when placed in the environment of the early embryo. The first indication that such participation could occur was provided by R. L. Brinster (*J. exp. Med.*, **140**, 1049-1056; 1974) who injected embryonal carcinoma cells from simple embryoid bodies from ascites fluid into 4 day mouse blastocysts of a different genotype and obtained one chimaeric animal. At a meeting held in May of this year at the Roche Institute, New Jersey, B. Mintz announced that she had successfully repeated this experiment, testing for chimaerism with several different genetic markers. In

Teratocarcinoma cells can develop normally

from Brigid Hogan

TERATOMAS and teratocarcinomas are rare tumours which arise in the gonads, and contain a whole variety of differentiated tissues of ectodermal, mesodermal and endodermal origin (such as skin, nerve, muscle, cartilage, gut and lung), mixed together in a disorganised mass. Although tissue culture lines of teratocarcinomas have been available for many years, it is only recently that technical progress in studies of the biochemical properties and *in vitro* differentiation of these cells has enabled their potential as a model for early mammalian development to be assessed (for a recent review, see Martin (*Cell*, **5**, 229-243; 1975). The paper by V. E. Papaioannou *et al.* in this week's *Nature* (page 70) now elegantly confirms and extends the key observations that, placed in the environment of the early embryo, teratocarcinoma cells are in fact able to participate in normal morphogenesis and differentiation.

The study of teratocarcinomas was first put on a modern scientific basis in the late 1950s by Leroy Stevens in the Jackson laboratory, who noticed that the 129/Sv strain of mice had a high frequency of spontaneous testicular

teratocarcinoma. Stevens and his colleagues showed that the tumours arose from germ cells in the testis and were retransplantable in either a solid or ascites form so long as nests of undifferentiated "embryonal carcinoma" cells were included in the inoculum; in the absence of these cells the transplanted tissue formed a slow-growing, benign, teratoma. The ascites form of the tumour consists of numerous small "embryoid bodies" floating in the ascites fluid; the simplest of these aggregates are made up of an outer layer of endoderm cells surrounding a core of embryonal carcinoma cells. This curious arrangement is in fact strikingly similar to the organisation of very early mouse embryos. At 4 days after fertilisation the normal blastocyst consists of a hollow trophoblastic vesicle enclosing a cluster of about 15 cells known as the inner cell mass (ICM); by 4.5 days the outer layer of the ICM differentiates into endoderm cells (which later form the extra-embryonic yolk sacs), while the internal cells go on to form ectoderm and mesoderm (the origin of the embryonic endoderm is at present unclear). Later work from a number of

It is now generally accepted that structures containing regions of heteroduplex DNA, with one strand of the duplex contributed from each parent molecule, are essential intermediates in the biochemically complex process of genetic recombination. During heterozygous crosses, the inclusion of a genetic marker within such a hybrid segment of DNA will mean the formation of a mismatched base-pair, or some other localised distortion of the DNA structure. It has been clearly shown in several systems that localised aberrations in DNA structure are subject to enzymic repair, by processes presumed to be analogous to the excision of pyrimidine dimers from ultraviolet-irradiated DNA. This repair of heteroduplex mismatches is believed to be a fundamental step in the recombination process and to be primarily responsible for the unequal segregation of the components of an allele-pair in the process of gene conversion.

Ahmad, Holloman and Holliday, in this week's issue of *Nature* (page 54) have now demonstrated that a nuclease from the smut fungus, *Ustilago maydis*, which had previously been implicated in genetic recombination, has the properties expected of an enzyme involved in the repair of mismatching heteroduplexes.

The enzyme, DNase I, purified from extracts of wild-type *U. maydis*, introduces single-strand breaks in linear, duplex and DNA molecules and reduces linear, single-stranded DNA to small oligonucleotides (Holloman, *J. biol. Chem.*, **248**, 8114; 1973). Ahmad *et al.* have now studied the activity of the nuclease towards linear duplex DNA

molecules containing local distortions, using heteroduplex molecules made from the separated strands of the DNA of bacteriophage SPP1. The state of the DNA can be sensitively monitored through its efficiency in the formation of infectious centres on transfection of competent cells of *B. subtilis*. Spatz and Trautner, who previously used this system to study the *in vivo* repair of heteroduplexes (*Molec. gen. Genet.*, **109**, 84; 1970),

Mismatched DNA and recombination

from a Correspondent

have shown that a single-strand nick inactivates the DNA molecule in the transfection assay.

During a standard exposure of the DNA to the nuclease, the extent of inactivation of heteroduplexes containing a single base-pair mismatch is about 6-fold greater than that of the homoduplexes. Heteroduplexes containing a small deletion on one strand are better substrates than those containing single base-pair mismatches. Thus the *Ustilago* enzyme is clearly able to recognise and preferentially attack those molecules containing distortions of the bi-helical structure. The nature of the biologically inactive products of the enzymic reaction was not investigated.

Well characterised nucleases from *Neurospora crassa* and *Aspergillus oryzae* have many similarities to the *Ustilago* enzyme, including the marked specificity for polynucleotides with disordered structures. The novel feature of the *Ustilago* enzyme is that

it is strongly implicated in genetic recombination. Double mutants of *Ustilago*, isolated by screening for deficiencies in both extracellular and intracellular deoxyribonucleases, show no detectable allelic recombination (Badman, *Genet. Res.*, **20**, 213; 1972) and much reduced DNase I activity (Holloman and Holliday, *J. biol. Chem.*, **248**, 8107-8113; 1973). The presumption that the deficiency in recombination and the loss of deoxyribonuclease activity are due to the same genetic lesions is supported by the finding that the two phenotypes are segregated together through meiosis.

The unusual specificity of these fungal nucleases makes them of more general interest as tools in molecular genetics. Their value is strikingly illustrated in a recent paper by Shenk, Rhodes, Rigby and Berg (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 989-993; 1975) who have used the *Aspergillus* nuclease, S1, to analyse mutations in the genome of the monkey virus, SV40.

Shenk *et al.* demonstrates that the S1 nuclease specifically hydrolyses linear heteroduplexes of SV40 DNA at the position of a single-strand nick, a small denaturation loop or even, in some cases, a single base mismatch. This property should have remarkable ramifications in molecular genetics, since it potentiates accurate localisation of genetic lesions in systems devoid of genetic recombination and of lesions that cause no phenotypic change. It should also facilitate the isolation of small, discrete segments of a genome in the many cases where the segment can be bounded by two suitable mutations. Satisfactory S1 nuclease is already commercially available!

the experiments reported by Papaioannou *et al.* three lines of teratocarcinoma cells cultured *in vitro* have been used as a source of donor cells and out of 121 animals born from inoculated blastocysts 11 showed evidence for chimaerism as judged by glucose phosphate isomerase isozyme, melanin and agouti markers. A proportion of the chimaerae from one cell line developed tumours, and some tissues (blood for example) do not appear to be chimaeric, but overall these results are very good evidence that at least some cultured teratocarcinoma cells are able to participate in normal development. As Papaioannou *et al.* point out, one implication of their experiment is that it should now be possible to select mutants of the cultured cells and use them to study metabolic cooperation and cell interaction during development. □

Evolution of tolerance to pollution

from Peter D. Moore

ALTHOUGH attention has recently been paid to the influence of pollutants on the structure and metabolism of whole ecosystems (for example Woodwell, *Science*, **618**, 429; 1970), the effects of pollution on species composition have been the subject of rather more research. Detailed observational studies, such as those of Hawksworth and Rose (*Nature*, **227**, 145; 1970) on epiphytic lichens have even made it possible to estimate ambient pollution levels from the species composition of communities. Such studies demonstrate that different species differ in their tolerance to a given pollutant, some responding at lower thresholds than others. But even within populations of a single

species, the presence of a pollutant in the environment selects against those individuals which are most sensitive to it. This has been demonstrated most effectively in the evolution of heavy metal tolerance in grasses near abandoned mines (Antonovics, Bradshaw and Turner, *Adv. Ecol. Res.*, **7**, 1; 1971), but an increasing amount of evidence suggests that air pollutants are exerting a similar influence.

The classic work of Kettlewell (see *The Evolution of Melanism*, Clarendon Press, Oxford, 1973) on the development of melanism as a response to air pollution by particulate matter, has demonstrated that such selection occurs among insect populations. The problem can be a complex one, however, as has been shown recently in the case of the two spot ladybird (*Adalia bipunctata*). Creed (*Nature*, **249**, 391; 1974) described the high incidence of melanism

in this species in areas of high smoke pollution. He showed a distinct negative correlation between the frequency of melanic forms and the distance from a smoke-emitting 'Phurnacite' plant, which produces smokeless fuel from coal, in the valleys of South Wales. Subsequently, however, an alternative explanation of this correlation has been put forward by Muggleton, Lonsdale and Benham (*J. appl. Ecol.*, **12**, 451; 1975). They have reconsidered the distributional evidence and come to the conclusion that melanism in the two spot ladybird is more closely related to the duration of sunshine in any area, which, in turn, is likely to be affected by smoke levels. They supplement this with experimental evidence that the temperature of the melanic form rises more rapidly when exposed to a given level of radiation, thus providing it with a metabolic advantage in conditions of low insolation.

Recent work among plants has concentrated upon sulphur dioxide as an air pollutant. About 6 million tonnes of SO₂ are emitted into the atmosphere over Britain each year; thus it constitutes one of our major air pollutants. Prolonged exposure to low concentrations of SO₂ (below about 15 p.p.h.m) does not seem to harm most plants, although depressed yield in ryegrass variety S23 (*Lolium perenne*) has been reported in experiments conducted at SO₂ levels below this (Bell and Clough, (*Nature*, **241**, 47; 1973). Since one of the most noticeable effects of SO₂ on plants is to cause increased stomatal opening (Unsworth, Bisco and Pinckney, *Nature*, **293**, 459; 1972), its deleterious influence may be indirect in allowing other toxic gaseous pollutants to enter the leaf tissue.

Sulphur dioxide pollution thus places a stress upon plant species and selects against those individuals in a population which are least tolerant. The effectiveness of this selective force has recently been demonstrated by the work of Taylor and Murdy (*Bot. Gaz.*, **136**, 212; 1975) in the vicinity of a coal burning power station in Georgia, USA. Seed was collected from a winter annual species, *Geranium carolinianum* at sites close to the station and at control sites some distance away, where SO₂ pollution was not a major stress. Nine weeks after germination, the plants were subjected to 12 h exposure to an atmosphere containing 80 p.p.h.m SO₂. Three days later leaf damage was assessed for each individual on the basis of mean percentage leaf area showing necrosis. The method of assay should overcome the differential absorption of SO₂ which occurs in leaves of different ages (Craker and Starbuck, *Environ. Res.*, **6**, 91; 1973).

Frequency distributions of plants with varying degrees of leaf damage

show a very considerable spread in all populations. It is to be expected that material derived from seed should exhibit wider variation than would the mature plants, since the latter would have been subjected to selection during establishment and maturation. Nevertheless, statistically significant differences were demonstrated between the mean leaf area damaged in populations near to and far from the source of SO₂ pollution. Differences were generally of the order of 10% less damage to the populations found close to the power station.

One can conclude, therefore, that there is a greater degree of tolerance to acute SO₂ pollution in the populations of *Geranium carolinianum* growing near a source of SO₂. It would be interesting to examine the relative tolerances of the populations to chronic exposure to lower SO₂ concentrations and to find out precisely what physiological adaptation is involved in the tolerance. This work indicates that very rapid selection (the power plant in question is just 31 years old) can occur amongst annual species, when subjected to a new environmental stress.

Transfer factor in viral infections

from Arie J. Zuckerman

THE transfer of cell-mediated hypersensitivity to tuberculin and streptococcal antigens was first achieved in man using blood leukocytes by Lawrence (*Proc. Soc. exp. Biol.*, **71**, 516; 1949). These observations in turn led to the finding that leukocyte extracts were as effective as viable cells for the transfer of delayed-type hypersensitivity. But these apparently innocent observations betray the controversy which surrounds transfer factor and which resulted in such sensational headlines as "Transfer Factor—science or magic?" (*New Scientist*, September 12, 1974) and "Transfer Factor—another scandal?" (*loc cit*, December 26, 1974). F. M. Burnet wrote recently:

"Widespread recent interest in the transfer factor of H. S. Lawrence and its possible therapeutic applications has not yet led to an interpretation of the phenomenon that can bring it into a satisfactory relationship with standard immunological theory. The specificity of transfer factor (TF) has been demonstrated in the sense that only a small proportion of the lymphocytes in a recipient can respond to a given TF preparation. The active agent in TF is known to be of low molecular weight (4,000 to 5,000), but its chemical nature and the basis of its specificity are unknown. Neither is anything known about the cell receptor that must be capable of specific reaction with a particular TF. An hypothesis is developed which sees the biological function of TF as to

recruit T lymphocytes to deal with situations such as delayed hypersensitivity or allograft rejection reaction for which there are inadequate numbers of immunologically reactive lymphocytes. Although the specificity of TF does not appear to be related to immunological specificity, it calls for a genetically based generation of diversity roughly analogous to that required in the development of the immune system." (*J. Allergy clin. Immun.*, **54**, 1; 1974)

The factual background to the controversy surrounding transfer factor is as follows. Transfer factor may best be described as a dialysable leukocyte extract that initiates and augments delayed-type hypersensitivity and cell-mediated immunity in normal persons, and Lawrence (*Adv. Immun.*, **11**, 195; 1969) considered transfer factor to be an antigen-specific polypeptide-poly-nucleotide molecule. Gottlieb *et al.* (*Lancet*, **2**, 822; 1973) found, however, that crude leukocyte dialysate from donors sensitive to *Candida* consisted, after concentration and elution from a column of Sephadex G 10, of three fractions, one of which was more active than the crude transfer factor. This fraction had a density of 1.47 g ml⁻¹ in caesium sulphate and the properties of a short polypeptide chain consisting of perhaps 12 amino acids joined to 3 or 4 RNA bases in a ratio of 2:1. The molecular weight of transfer factor is less than 10,000, probably 2,000–4,000. It is resistant to trypsin and mammalian DNase and RNase and the ultraviolet spectrum indicates the presence of polypeptide moieties. It is not related to any immunoglobulin class and it is non-immunogenic in man or animals. The present concept of transfer factor is therefore that of a soluble indicator of cellular immunity, for example an informational polynucleotide or a specific gene derepressor, which instructs previously uncommitted lymphocytes to become specifically sensitised and thereby transfers the state of delayed hypersensitivity. It has been suggested that cellular cooperation between T lymphocytes, B cells and macrophages may be associated with lymphokine factors and transfer factor for the induction of delayed hypersensitivity and antibody synthesis or in cytotoxic activity against cells with viral, tumour or histocompatibility antigens (Playfair, *Clin. exp. Immun.*, **8**, 839; 1971). In fact, Dumonde and Maini (*Clin. Allergy*, **1**, 123; 1971) suggested that, in the presence of a specific antigen, transfer factor induces non-sensitised lymphocytes to produce lymphokines.

Clinically, transfer factor activates uncommitted and non-sensitised lymphocytes of the recipient so that new clones of antigen-specific cells are produced. Besides conferring specific skin sensitivity, these cells may also

produce interferon and other biologically active products which, as a result of non-specific stimulation, may contribute to the overall pattern of the cellular immune response in the recipient. It should be noted that transfer factor seems to be exceedingly potent; the amount of factor required to confer systemic skin reactivity on a normal recipient is the extract of only 0.1 ml of packed leukocytes. Reactivity to the other antigens to which the donor was sensitive, such as *Candida*, mumps, streptococcal and other antigens, is transferred simultaneously. But transfer of the capacity for antibody formation does not occur.

The clinical use of transfer factor is based on the view that depressed cell-mediated immunity is of pathogenic importance in various disease states and that, by reversing the deficiency, transfer factor can ameliorate the disease process. This has been tested in severe infections caused by mycobacteria, fungi and viruses, particularly in patients with defective lymphocyte responsiveness either *in vivo* or *in vitro*. On the same basis it has been possible to correct certain primary immunodeficiency states in some patients exhibiting selective depression of lymphocyte function.

Patients suffering from a number of infectious diseases, including mucocutaneous candidiasis, coccidioidomycosis, leprosy and a number of viral infections, have been treated with transfer factor. The patients responded to treatment with transfer factor obtained from donors in whom skin tests to the particular antigen were strongly positive. Successful outcome has been reported in a few patients with generalised vaccinia, herpes zoster, neonatal herpes virus infection, measles "giant-cell" pneumonia and subacute sclerosing panencephalitis.

Spitler and colleagues (*Clin. Immunobiol.*, edit by Bach, F. H., and Good, R. A., Academic Press, 1974, p. 153) treated a number of patients with verruca vulgaris, an example of a chronic viral infection, with transfer factor pooled from normal donors presumed to have immunity to this virus. Clinical improvement occurred in only one patient after several months of therapy so that the improvement may be quite unrelated to the use of transfer factor. A note of caution was added recently by Stevens and his associates (*Clin. exp. Immun.*, 21, 520; 1975). Dialysed transfer factor, prepared from the leukocytes of a donor whose warts had undergone spontaneous regression, was used in the treatment of a child with Wiskott-Aldrich syndrome, a congenital immune deficiency. The child then had a spontaneous regression at multiple areas with warts. A similar relationship was observed in a pilot

study in four otherwise healthy patients. But a randomised double-blind study of thirty patients failed to confirm a causal relationship between therapy with transfer factor and wart regressions.

Attention has also been directed recently to persistent infection with hepatitis B virus. Kohler and his colleagues (*Clin. Immun. Immunopathol.*, 2, 465; 1974) treated a healthy persistent carrier of hepatitis B surface antigen with lymphocytes from an individual who had recovered eight months previously from hepatitis B. Within 24 h there was an increase in the titre of antigen and of serum aspartate transaminase levels, an indication of liver damage. It was suggested that the recipient's liver cells were transiently injured by a cell-mediated immune response, which was insufficient to terminate the infection. Transfer factor prepared from the same donor had no effect on the patient's liver function tests. The biological activity of this material, however, was not evaluated and it may have been inactive. Another patient, an infant who acquired hepatitis from her mother at birth, was given transfer factor prepared from the mother's lymphocytes. On two occasions the preparation caused a prompt, moderate increase in both the antigen and transaminase levels. Subsequently, the liver function tests became normal and the titre of hepatitis B surface antigen was reduced by 95%. It is generally considered that cell-mediated immunity is important in terminating hepatitis B infection. Temporary falls in the levels of hepatitis B antigen have also been reported after the use of transfer factor in a few patients with antigen-positive chronic active hepatitis. Jain and his colleagues reported at the meeting of the British Society of Gastroenterology held in Oxford in September, 1975, the results of treating three patients with "specific" transfer factor prepared from subjects recently recovered from hepatitis B infection. One patient with chronic active hepatitis, and cirrhosis associated with antigenaemia showed no response to transfer factor prepared from a normal blood donor but "specific" transfer factor increased the number of T lymphocytes; a second similar patient also showed no response to "normal" transfer factor but "specific" transfer factor increased transiently the serum aspartate transaminase levels suggesting stimulation of cell-mediated immunity with a resulting hepatitic reaction. The third patient suffered from antigen-positive active chronic hepatitis complicated by primary liver cancer; he was receiving corticosteroid therapy and did not respond to transfer factor. It was concluded that although there was no

Earthquakes without precursors?

from Peter J. Smith

SOME shallow earthquakes are known to be preceded by significant decreases in P and S wave velocities (V_p and V_s) and the seismic velocity ratio V_p/V_s . One of the problems in using these effects as a basis for earthquake prediction, however, is that not all shallow shocks appear to give such warning. Reasons proposed so far for the lack of premonitory effects in certain cases range from failure to measure V_p and V_s at the appropriate times to possible variations of the V_p , V_s and V_p/V_s decreases when measured in different directions with respect to the trend of elongated cracks in the surrounding rock. But two new experiments carried out by Wang *et al.* (*Geophys. Res. Lett.*, 2, 309; 1975) now suggest that the apparent presence or absence of precursory phenomena may depend not simply on observational arrangements but on something much more fundamental.

In the first experiment, a block of dry 'Westerly' granite with an imposed normal load was subjected to an increasing shear load until fracturing occurred. Both V_p and V_s for waves travelling normal to the shear plane decreased before rupture, and dilation took place in the same direction. V_p/V_s decreased from a 'normal' value of 1.7 to 1.4 in line with previous field and laboratory observations. In the second experiment, the granite block was first sliced in half and then subjected to normal and shear stresses to give stick-slip motion. But no V_p and V_s changes were observed either before or during sliding, and there was no dilation against the normal pressure.

Wang and his colleagues conclude from this that there must be appreciable variations in the faulting process leading to shallow earthquakes. The degree to which V_p and V_s decrease will then depend on the extent to which faulting is associated with dilation and fracturing. The implication is that prediction based on precursory seismic wave velocity variations will not be possible even for all shallow earthquakes.

alteration in the serum titres of hepatitis B surface antigen further trials with transfer factor seem justified.

Finally, Bullock *et al.* (*New Engl. J. Med.*, 287, 1053; 1972) pointed out that there is a risk of transfer factor exciting generalised hypersensitivity reactions to disseminated microbial or other antigens and that, paradoxically,

Just under two decades ago, four groups reported almost simultaneously the presence of antibodies against DNA in the serum of patients with systemic lupus erythematosus (SLE). While early studies used double stranded (ds) DNA, it was found that many, if not all, SLE sera also reacted against single stranded (ss) DNA. Indeed it is now apparent that a heterogeneous collection of anti-DNA antibodies exists, binding to a variety of sites on the polynucleotides (Arana and Seligmann, *J. clin. Invest.*, **46**, 1867; 1967; Cohen, *et al.*, *Clin. exp. Immun.*, **8**, 551; 1971). During the past six years, the measurement of DNA antibodies by the ammonium sulphate precipitation technique (Farr) has become a standard clinical tool in the diagnosis and management of SLE. While antibodies against ds DNA occur almost exclusively in SLE, anti-ss DNA antibodies are not specific for this disease, occurring in rheumatoid arthritis, drug-induced lupus and a variety of other conditions, as well as in some normal sera.

The agent most commonly used in DNA binding tests is ^{14}C -labelled bacterial or mammalian double stranded DNA, and therein lies the main obstacle to standardisation of results. It has proved difficult using these preparations to assess the contribution to 'native' DNA binding of single stranded regions in the molecule. A recent study by Samaha and Irwin (*J. clin. Invest.*, **56**, 446; 1975) used a variety of techniques in an attempt to further characterise the antigenic regions in mammalian DNA. Using MAK chromatography followed by enzyme removal of single stranded regions, they showed that purified mammalian DNA is a mixture of almost homogeneous ds DNA, and ds DNA with significant single stranded regions. The latter were

found to be rich in thymine, complementing the earlier studies of Koffler and his colleagues (*J. exp. Med.*, **134**, 294; 1971) who showed that sera with antibody to ds DNA are inhibited by the synthetic single stranded polymer poly(dA), less so by poly(dT), and

Antibodies to polynucleotides in SLE

from Graham R. V. Hughes

least of all by poly(dC).

These studies suggest that some antibodies in SLE are directed at the ends of the molecule containing short thymine-rich single stranded portions and possibly also at areas of bifurcation between ds DNA and AT-rich single stranded regions in ds DNA. Thus it seems that the variable binding activity reported with various SLE sera may have more complex explanations, not simply reflecting specificity towards ss or ds nucleic acid, but possibly also pointing to differential binding of antibody to specific regions of structurally heterogeneous DNA. Two possible ways of bypassing the problem of ss DNA binding might be the use of circular ds DNA (such as SV40 DNA) or the use of synthetic self replicating polymers such as poly (dAT). Neither of these antigens has yet received adequate clinical trial. In the meantime, the clinical significance of slightly raised DNA binding values in some non-SLE sera remains uncertain.

Apart from clinical considerations, delineation of the various polynucleotide antigen-antibody systems has more than academic interest. The renal disease in SLE results largely from immune complex deposition, with DNA-anti-DNA antibody complexes being of considerable patho-

logical importance. Less clear, however, is the relative contribution and possible interaction of the various polynucleotide complexes. Discrepancies appear to exist, for example, between the presence of anti-ss DNA antibodies in rheumatoid arthritis and scleroderma, and the lack of glomerular immune complex deposition in the majority of patients with these diseases.

One of the earlier known manifestations of circulating immune complexes in SLE was the presence of cold precipitating globulins (Christian, Hattfield and Chase, *J. clin. Invest.*, **42**, 823; 1975) and in a recent study, Winfield, Koffler and Kunkel (*J. clin. Invest.*, **56**, 563; 1975) have returned to cryoprecipitation in an attempt to determine the specific concentration of anti-polynucleotide antibodies in cryoprecipitates relative to serum levels. Using haemagglutination techniques, they found that antibody against ds DNA and ss DNA were both enriched up to a 100-fold in some SLE cryoprecipitates, as compared with serum. Antibody to ribonucleoprotein (thought to be associated with milder or absent renal disease) was only occasionally concentrated in cryoprecipitates. In contrast, anti-ds-RNA, which was commonly detectable in high titre in SLE serum, was only minimally concentrated, and only in a minority of precipitates. These data are in broad agreement with earlier elution studies of SLE glomeruli, where again anti-ds-RNA was not detected.

The findings again indicate that different anti-polynucleotide antibodies have differing pathogenetic properties. While this has, in essence, been known for over a decade, the dissection of these antigen-antibody systems appears now to be gaining momentum, the clinical goal being more precise management of SLE.

it may be necessary to use anti-inflammatory drugs during the early stages of treatment. In immune deficiency states there is a suggestion that uncontrolled lymphoid cell proliferation and autoimmune disease may be additional complications (Gelfand, E. W. *et al.*, *New Engl. J. Med.*, **289**, 1385; 1973, and Ballou, M. *et al.*, *J. Pediatr.*, **83**, 772; 1973). Lawrence (*Clin. Immunobiol.*, *loc cit*) noted that "therapy with transfer factor (TF_D) as presently employed is empirical, since the precise mechanism via which this small molecule confers or uncovers a specific antigen-receptor site on the recipient's circulating lymphocytes remains to be clarified."

Immunotherapy with transfer factor for the persistent carrier of hepatitis B

surface antigen or antigen carriers with chronic liver damage requires a cautious and carefully considered approach. The safety and effectiveness of such preparations should be evaluated as far as possible in animal model systems (*WHO Techn. Rep. Series*, No. 570, 1975). There is also, of course, a need to establish provisional standard reference preparations of transfer factor and studies on specificity are urgently required.

Carbon-13 NMR of proteins

from J. Feeney

IMPROVEMENTS in NMR instrumentation over the last few years have

largely overcome the sensitivity problems which had previously retarded ^{13}C NMR studies of medium-sized molecules (molecular weight >1000). By using a combination of Fourier transform and proton noise decoupling techniques, high sensitivity natural abundance ^{13}C spectra can now be obtained for small peptides such as oxytocin, angiotensin and LHRH in which each carbon features as a single sharp resonance signal. For larger peptides there is considerable overlap of the ^{13}C spectral lines and when a small protein such as lysozyme is examined relatively few single ^{13}C resonances can be resolved.

Professor A. Allerhand and his co-workers (Oldfield *et al.*, *J. biol. Chem.*, **250**, 6381; 1975) have now reported

methods which allow them to detect most of the aromatic quaternary carbons in small proteins. By using a proton noise decoupling field slightly off-resonance for optimum proton decoupling only the quaternary carbons are efficiently decoupled and the sharp signals from these carbons can then be separated from the very broad bands from proton-bearing carbons using convolution difference methods (Campbell, *et al.*, *J. Magn. Res.*, **11**, 172; 1973). The resulting spectra feature only the quaternary carbon signals. Allerhand and coworkers have applied these techniques to several native proteins (lysozymes, cytochromes *c* and myoglobins) and using concentrated aqueous solution (10–20 mM) in the 20 mm diameter sample tubes at 15.18 MHz they are able to obtain high quality spectra in ~5 h of accumulation time.

Many assignments of the signals could be made by consideration of chemical shifts, relaxation behaviour in H_2O and D_2O , selective proton decoupling experiments, pH dependence, deuterium isotope effects on chemical shifts and line broadening induced by bound lanthanide ions. For hen egg white lysozyme the γ -carbons of the 6 Trp residues have chemical shifts over a range of ~5 p.p.m.; when the protein is denatured with guanidine the signals coalesce into a single absorption band. In the ^{13}C spectrum of horse heart cytochrome *c* all 18 non-protonated aromatic carbon signals are resolved. When the diamagnetic forms of haem proteins such as horse heart ferrocytochrome *c* are examined, the haem carbons are also detected in the spectra but these disappear in the spectra of the paramagnetic form. In mixtures of ferro- and ferricytochromes *c* there is fast electron transfer which produces exchange effects in the averaged spectrum such that a 1:1 correspondence between non-protonated aromatic carbon signals from the two forms can be established.

Clearly one can use the assigned ^{13}C signals in the various proteins to monitor protein unfolding, ionisation states of specific residues and interactions between the protein and other molecules. It should be borne in mind, however, that studies of ^{13}C at natural abundance (1.11%) have the disadvantage that they require large amounts (50 mM per sample) of soluble protein.

An alternative approach is to enrich the protein selectively with ^{13}C -labelled amino acids. In this way, the concentration and solubility requirements (<1 mM solutions can be examined) are reduced and a much wider range of proteins can be studied. Several workers have demonstrated that ^{13}C -labelled amino acids can be incorporated into

bacterial proteins from auxotrophic organisms. An elegant example is found in the work of Hunkapiller and coworkers (*Biochemistry*, **12**, 4732; 1973) who isolated α -lytic protease from *Mycobacter* 495 grown on a medium containing all the naturally occurring amino acids including 2- ^{13}C -labelled histidine. There is only a single histidine residue in α -lytic protease (His-57) and thus there is no assignment problem for the intense signal obtained in the protein ^{13}C spectrum. The C-2 carbon is coupled to the directly bonded proton and the measured $^2J_{CH}$ coupling constants are characteristic of the ionisation states of the imidazole ring ($^2J_{CH}$ =219 Hz when the ring is protonated and $^2J_{CH}$ =206 Hz for the unprotonated ring). Thus they were able to show from such measurements that the *pK* of His-57 is very low in the enzyme (*pK*<4), a fact which has important implications in understanding the mechanism of action of serine proteases.

Usually the incorporated labelled amino acid occurs at several different sites in the protein and one is faced with the difficult task of assigning the ^{13}C signals. A general solution to the problem of observing and assigning ^{13}C signals from proteins will be available when semisynthetic proteins can be made by incorporating synthetically prepared fragments containing several different labelled amino acids in a region of interest in the protein structure.

Tumour viruses discussed at Copenhagen

from Ian Macpherson

The Seventh International Symposium on Comparative Research on Leukaemia and Related Diseases was held in Copenhagen on October 13–18.

ONE of the most important issues raised at the meeting concerned the true nature of the C-type viruses apparently isolated from the leukocytes of a human case of acute myeloid leukaemia by Gallagher and Gallo (*Science*, **187**, 350; 1975) also from the bone marrow cells of a child with lymphosarcoma by Nooter *et al.* (*Nature*, **256**, 595; 1975) and from human embryo lung cells passaged in culture by Panem *et al.* (*Science*, **189**, 297; 1975). These viruses were recognised as typical C-

type particles like those known to cause animal leukoses and were found to have antigenic and reverse transcriptase affinities with simian sarcoma virus (SiSV). These relationships were not considered to be surprising or even unexpected but what raised a few eyebrows at the meeting were the observations made in several laboratories using competition radio-immunoassay analysis and molecular hybridisation, that both Gallagher and Gallo's and Panem's isolates each contain two distinct viruses with uncomfortably close affinities to SiSV and the baboon endogenous virus. At present it is not possible to say who got what from where and when. The forthcoming conference of the Virus Cancer Program at Hershey may help clarify these uncertainties.

Regardless of how these questions are answered there is evidence from other sources suggesting that man commonly encounters viruses antigenically related to the murine, feline and/or simian agents. H. W. Snyder (Sloan-Kettering, New York) found that most, if not all, adult human sera contain antibodies that combine with polypeptides from these C-type RNA tumour viruses. Placental cord sera are negative and sera from leukaemic patients reacted like normal sera. These results suggest that a virus or viruses with antigenic specificities common to the animal C-type viruses used as probes in this study are widespread in the human community. R. S. Metzgar (Duke University Medical School) reported antigenic relationship between RNA tumour viruses and surface antigens of leukaemic cells in cytotoxicity tests. He has shown that human leukaemic myeloblasts have antigens related to the p30 and gp70 proteins of Friend mouse leukaemia virus. R. A. Lerner (Scripps, La Jolla) has found that gp70 has a remarkable pleomorphism in the mouse. By immunofluorescence he has shown that this component of mouse leukaemia virus surface coat is also present in mouse lymphoid and epithelial cells and in the secretions of the epididymis. The system offers an excellent model for the study of host control of this gene's expression.

An interesting observation that may provide at least a partial explanation why C-type viruses are not as readily detectable in man compared with other animals also came from studies carried out at Scripps: F. C. Jensen provided evidence that human complement, without the intervention of antibodies, was capable of lysing a wide range of C-type viruses. This may provide man with an important line of defence against infection with viruses of this type.

P. H. Duesberg (University of

California, Berkeley) described further progress his group has made on the mapping of avian tumour viruses. The 30-40S RNA, which they have shown to have a 3' poly(A) end, was fragmented with alkali. Fragments with the poly(A) tag were separated according to size and the location of a particular oligonucleotide relative to the poly(A) end was deduced from the smallest piece that could be found. Different virus strains were found to share certain oligonucleotides and these were in approximately the same linear location. Comparisons between transformation defective, and virus envelope defective mutant viruses with their respective wild-type progenitors have shown that the transformation gene(s) (the *onc*) is located near the poly(A) end and that the information for the virus envelope (*env*) is nearby. The continued application of this type of analysis in the hands of such able interrogators should eventually provide a complete picture of the genetic map of these viruses.

Another intriguing probe into the avian sarcoma viruses and their dealings with the cell was described by D. Stehelin (University of California Medical Center, San Francisco). By making a radioactive DNA complementary to the whole RNA genome of a transforming virus and "absorbing" it with the RNA of a mutant virus without the transforming functions they were able to make a cDNA *onc* gene probe. Leukosis viruses and transformation defective mutants lacked these sequences. Uninfected cells of a variety of avian species including duck (which so far has not been found to contain an endogenous virus) contain the nucleotide sequences of the probe in their DNA but do not transcribe the gene into RNA. However, their finding that methylcholanthrene induced tumour cells from the quail contained homologous RNA to their probe is of considerable interest and hints at a link between chemical induction of tumours and the activation of "viral" genes.

The small DNA tumour viruses were brought back into the arena by H. zur Hausen (Universität Erlangen-Nürnberg). He has shown that human genital and laryngeal warts which sometimes undergo malignant transition were not found to have any measurable homology with a complementary RNA prepared from plantar wart DNA. This suggests that there are a variety of papilloma viruses in man, some of which may be responsible for malignant tumours. This field may be due for a revival especially since there has been a recent report by Eisinger *et al.* (*Nature*, **256**, 432; 1975) that human wart virus can be grown in tissue culture. □

Cell motility moves ahead

from E. W. Taylor

The most recent conference in the Cold Spring Harbor series on Cell Proliferation, entitled Cell Motility, was held on September 9-14.

A KNOWLEDGE of how cells move and how their movements are controlled is necessary for an understanding of a variety of biological processes including embryological development, the wiring of the nervous system, and the invasive transformation of the cancer cell. During the past few years the number of papers devoted to some aspect of cell motility has increased enormously. The conference was a largely successful effort to establish communication among the practitioners in this diverse field. The topics included muscle contraction, non-muscle actomyosin and streaming, structure and function of animal and bacterial flagella, chemistry and assembly of microtubules and mechanisms of mitosis.

Three main classes of motile systems can be distinguished on the bases of protein composition and energy transduction mechanism, the bacterial flagellum, the eukaryote flagellum, the actomyosin system of muscles and cytoplasmic streaming.

The mechanism of bacterial flagella movement has long been a puzzle since the flagellum consists of a single protein with no known enzymatic activity. It has been shown that the flagellum does not propagate helical waves but rotates, driven at the base by a structure in the membrane. The sense of rotation can be reversed, producing swimming in the opposite direction if a single flagellum is present or preventing swimming by untwisting the flagella bundle. A physical analysis of the motion was presented by H. C. Berg (University of Colorado) and the experiments establishing the rotation mechanism and the properties of motility mutants were discussed by M. Simon (University of California, San Diego) and J. Adler (University of Wisconsin). D. E. Koshland (University of California, Berkeley) described elegant mixing experiments which established the point that the chemotactic response was elicited by a change in concentration with time rather than a spatial concentration gradient across the bacterium. These studies lead to a simple model of chemotaxis which should be widely applicable to the movements of animal cells. The frequency of reversal of the motor is reduced by an increase in concentration of attractant. In simplest terms, if a

bacterium is moving up a concentration gradient, motion in this direction will persist longer. If it moves down the gradient it will stop in a shorter period of time and try a new direction. As a result the step lengths in a random walk are biased in the direction of the concentration gradient.

The sliding filament-swinging cross bridge mechanism of muscle contraction has been firmly established and a reasonable correlation has been made with corresponding biochemical events in ATP hydrolysis by actomyosin. In recent years actomyosin has been isolated from a variety of tissues and organisms (brain, platelets and fibroblasts of vertebrates, slime mould, amoebae and algae). It is very likely that actomyosin is responsible for cytoplasmic flow and actomyosin-containing extracts of amoebae, studied by D. L. Taylor (Harvard University), mimic the flow properties of the intact cell. Several laboratories have studied the properties of cellular actin and myosin and a pattern is beginning to emerge. While the actin and myosin are similar to but distinguishable from their muscle counterparts, the regulation of ATPase activity and aggregation into gels depends on factors which are different or absent from the muscle system. Preliminary characterisation of protein factors which regulate actin polymerisation, aggregation of actin into bundles and formation of gels has been achieved in slime moulds by S. Hatano (Nagoya University), M. R. Adelman (Duke University Medical Center), J. A. Spudich (University of California, San Francisco), in amoeba by T. Pollard (Harvard Medical School) and in macrophages by T. P. Stossel (Children's Hospital Medical Center, Boston). Regulation of ATPase activity by Ca requires a protein factor which is distinctly different from troponin or a myosin light chain. Recent studies on this factor in amoeba and macrophages were reported by Pollard and Stossel respectively. The field has passed out of the stage of counting up the number of systems which contain actomyosin to go on to the determination of the special properties of actomyosin systems which are peculiar to cytoplasmic flow.

Bundles of actin filaments are common cellular components but under some conditions a lattice is formed with α -actinin (a muscle protein) anchoring the crossover points of the lattice. Fluorescent antibody staining developed by E. Lazarides (Cold Spring Harbor) has enabled these complex networks to be made visible. At present we are suffering from a surfeit of actin filament bundles which seem to be present even in the mitotic spindle. These studies have reopened the question of whether actomyosin participates in

chromosome movements (the question was not settled). The possibilities of artefacts in preparing cells for antibody or heavy meromyosin (HMM) labelling was keenly discussed and although there is growing evidence for the presence of actin, myosin has not been demonstrated. The studies of L. G. Tilney (University of Pennsylvania) have shown that actin is present on its own in the acrosome bodies of sperm from a variety of species and is driven into the egg by polymerisation or uncoiling. Thus the presence of actin in a cell organelle need not imply the presence of myosin as well.

Some three years ago, R. C. Weisenberg (Temple University, Philadelphia) developed a method of polymerisation of tubulin into microtubules. This work stimulated an intense study of the mechanism of microtubule polymerisation and the search for microtubule organising centres. Condensation of tubulin into outer doublets of flagella and asters of the mitotic apparatus has already been described and evidence was presented (by two different laboratories) for the chromosome kinetochore as an organiser. Microtubule polymerisation has turned out to be a complex process, more like virus assembly than the type of spontaneous polymerisation that occurs with fibrous proteins (actin for example). The formation of a ring structure by a protein factor or possibly polyvalent ions, the use of the ring as a nucleating centre, the participation of high molecular weight factors, and enzymes (transphosphorylase and possibly a protein kinase), the requirement for nucleotide triphosphate hydrolysis, as well as three different polymerisation models (H. P. Erickson-M. Kirschner (Duke University), G. G. Borisy (University of Wisconsin), M. Jacobs *et al.* (King's College, London)) were discussed. While it is clear that rapid progress is being made on this problem, more rigorous purification of the system is necessary before the discrepancies between different groups can be resolved. □

Transient observations of X-ray sources

from John Gribbin

At the Royal Astronomical Society meeting on October 10 the nature of the transient X-ray source Ariel 0620-00 was discussed.

THE discovery of the source early in August was described by M. Turner (University of Leicester). There was a

rapid rise in intensity, with one slight hiccup, which made A0620-00 three times brighter than Sco X-1 by the middle of the month (see *Nature*, 257, 656, 657; 1975). The hiccup, or 'precursor peak' seems to have marked a change in the energy production mechanism, with a fall in temperature preceeding the most rapid period of increasing brightness, and with the bulk of the increase occurring at low energy (below 10 keV) making this a very soft source. No lines have been found in the spectrum, although searches have been made especially at frequencies appropriate for ionised silicon and sulphur, and there is no evidence of polarisation at what Turner described as "the lowest upper limit yet achieved in X-ray astronomy" and there is no evidence of periodic variations anywhere in the range 0.2 ms to 2 d.

But there are some positive interpretations of the X-ray evidence, not least a SAS 3 measurement of the hydrogen column density to the source which implies a distance of 3 kpc if all absorption is interstellar, suggesting an absolute luminosity of 10^{39} erg s⁻¹.

A0620-00 has also been detected at radiofrequencies and identified with an optical star (see *Nature*, 257, 659; 1975). R. Davis (Jodrell Bank) described radio observations both at Manchester and elsewhere which help to provide a very accurate position in right ascension, although the declination is less well determined because of the inconvenient position of the object for such measurements. The source is variable, and declined from peak intensity after August 18 with an *e*-folding time of 5 d, considerably faster than the equivalent X-ray decline (*1/e* fall off over 30 d). Assuming the outburst began on August 3, the radio data can be fitted by a T^{-3} power law.

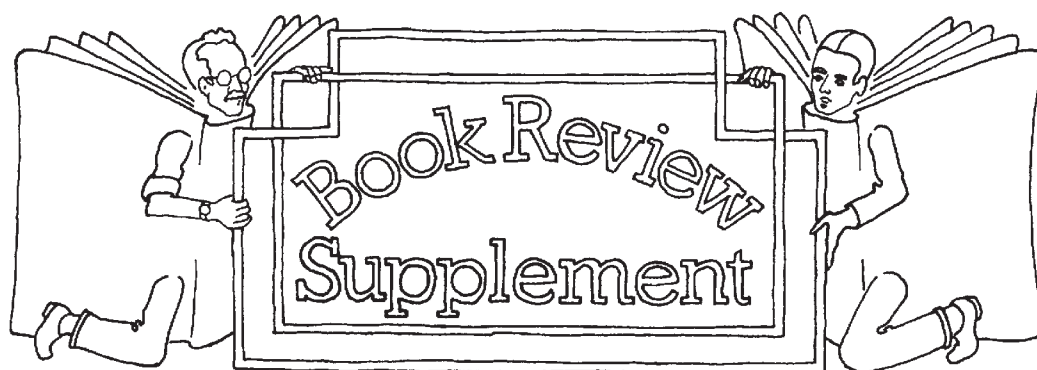
The optical observations were described by M. Penston (Royal Greenwich Observatory), who preferred to call the event Nova Monoceros 1975 but agreed that he was indeed talking about the same thing. There seems no doubt at all about the identification of the optical and X-ray novae, and comparison with Palomar Sky Survey plates shows that during the outburst the source increased from a magnitude of 20.5 to 11.5, a brightening which is on the high side for dwarf novae but not unprecedented. The Palomar plates also show that the star was very red before the outburst, suggesting that it may be a red dwarf only some 500 pc away or a giant at the implausible distance of 15 kpc which, in the direction of Monoceros, would place it not only well out of the Galaxy but far above the galactic plane. At optical frequencies, the fade of the source has

been little slower than in the X-ray band but faster than at radio frequencies, indicated by measurements up to a few days before the RAS meeting.

For the modellers, one of the most significant contributions comes from a search of Harvard plates which show a previous outburst in 1971, reaching 12 mag. This strongly implies the presence of circumstellar material leftover from that event, suggesting that the SAS 3 distance must be an upper limit and pinning the object down clearly as a red dwarf probably closer than 1 kpc.

A. Fabian (Institute of Astronomy, Cambridge) took up this theme in presenting a model of A0620-00. Pointing out that this is the first optical identification of a transient X-ray source, he made a leap which perplexed some optical astronomers—but not the X-ray specialists—by assuming the now basic model of a binary source with mass transfer from the visible star onto a compact companion, probably a neutron star. The probability of seeing an eclipse on such a model is only 12%, so it is not surprising that none is seen in this source, and the increase in brightness of the dwarf is caused by the heating effect of X-radiation from the companion on one face of the larger star. The fact that novae seem to recur in this system encouraged Fabian to make a comparison with the recurrent nova WZ Sge, itself a similar binary system, and although this detailed model is unlikely to fit all the transient X-ray sources it is interesting that a very similar model has already been proposed for the archetypal X-ray source Sco X-1, again drawing a comparison with WZ Sge.

This raises the question of just how different the transient X-ray sources are from the rest of the family. All of the known X-ray sources that are not supernova remnants show very erratic behaviour with flares and quieter periods, and if Sco X-1 can be explained, after more than 10 years observation, by a model similar to that appropriate for a newly discovered source which flared into prominence for only a few weeks it seems that the differences may well be of degree rather than of kind. The observations themselves are far from continuous, and the label hung upon any particular source can depend to a large extent on the state it happened to be in when it was first noticed. Perhaps the main lesson to be learned from the story of A0620-00 so far is that all X-ray binaries are variable, but some vary more than others, and that rather than talking about "observations of transient X-ray sources" we would do better to talk about "transient observations of X-ray sources". □



Michael Shillaker

WHEN asked to write on the books that scientists were reading when I was young, I thought at first that it would be impossible to make anything of interest out of what I personally had been reading. There are, however, some points which may have some wider relevance. We will be dealing with the years 1925–30, when I was 20 to 25. That was just before many things happened, which we now accept as in the very nature of things.

When I ask myself "what was that time *before*?", I have to think of a very wide range of different topics. At that time it seemed absolutely natural that a young man of my age, interested as all such people are, in the nature of the world in which he was beginning to find his feet, should expect to find out about a great many different aspects of it. Not only his own science, but several sciences connected with it, even if only loosely. My 'official' interest was in what fossils can tell us about the processes of evolution. There was not very much general reading in that connection. That was *before* the development of mathematical neo-Darwinism, although J. B. S. Haldane was beginning to do some elementary sums about the selection of single genes, which he, rather sensibly, treated rather as undergraduate *jeux d'esprits* than as the profound contributions to fundamental biology which people later—and still—pretended them to be.

It was also absolutely natural to have interests in philosophy, poetry, even painting, and to allow them to show. This was well *before* there was considered to be any firm dividing line between the natural or the moral sciences, or even between those and

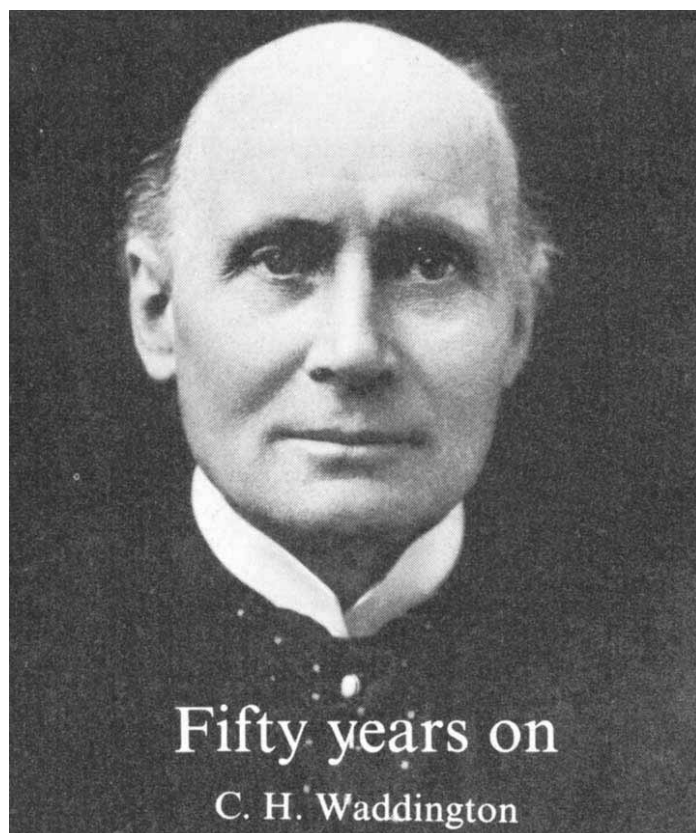
the arts. It is in these areas that some of the *befores* are most difficult to clarify. Eliot's *The Waste Land*, and some of Ezra Pound had already been published, but it was *before* their influence had changed the whole direction of English poetic taste, at least as far as I was concerned; at that time, I was still reading English poets of the

later became almost the hallmark of the profession. But this was *before* the influx of that remarkable succession of Viennese intellectuals which have dominated British philosophy since the early thirties—Wittgenstein, the first, best, and only one to recognise for himself that he had been talking nonsense and to spend the rest of his life unsuccessfully trying to get it right; followed by Schlick and Carnap, with their first major British follower, Ayer; then Popper, and Lakatos and now Feyerabend, whose book, *Against Method* (NLB London, 1975), should be the expiring gasp of the lineage of thinkers who set out to describe the definitive method of science in terms of logical algebra.

The two people I refer to, just *before* these, were I. A. Richards, with his *Principles of Literary Criticism* followed a few years later by *Practical Criticism*, and C. K. Ogden, who collaborated with Richards in *The Meaning of Meaning*, which was much more philosophy than criticism. Ogden also introduced us to, at least some, of the problems of linguistics, first from his own viewpoint, by the attempt to develop a "minimum effective language" (Basic English), but

later using the fashionable Viennese ideas, as the first translator of Wittgenstein's *Tractatus*. I doubt if, today, you would find anywhere the intimate interplay between poetry, philosophy and the foundations of science which Ogden and Richards displayed.

The other main influence on me as a young man just growing up as a scientist, was that of the philosopher, A. N. Whitehead, the writings of whom some younger biologists seem to associate with molecular biology, or the even more recent influences of Levi-



A. N. Whitehead

generation of Wilfred Owen, Edward Thomas, Gordon Bottomley, Walter de la Mare, the Sitwells and the American experimentalists and imagists, such as e e cummings, Laura Riding, Hart Crane, John Crowe Ransom and H.D.

But most important of all, certainly for my future development, were books by two people who were closely associated and who brought literary criticism and philosophy very near together. The official philosophers in Cambridge, such people as Broad and Moore, had already some of that remoteness which

Straus and Chomsky. He was the senior author, with Bertrand Russell, of the great tome on *Principia Mathematica* which laid the foundations of philosophy in the mode of logical algebra. His later treatment seems to me to border on the fantastic. It was Russell who persisted in the directions adumbrated in the *Principia*, and that work seems nowadays to be considered virtually his alone. In fact, Whitehead scarcely gets mentioned in most modern outlines of philosophy except as Russell's co-author in that work.

Admittedly, Whitehead did not make it easy for readers of later generations who were in a hurry to catch up. For instance, R. F. Atkinson (*Twentieth Century Mind*, 2, Oxford), dealing with British Philosophy, from 1918-45, finds little more to say than: "His major metaphysical works are *Science and the Modern World* (1925), *Adventures of Ideas* (1933), *Process and Reality* (1929)—the latter very difficult work being the most complete statement of his views—...and a vast expenditure of time is needed to come to terms with his system. His opponents therefore ignore him instead of criticising him in detail". This is as though some one felt he was duty bound to assess the literary worth of James Joyce, and decided, after plunging into *Finnegan's Wake* for half an hour, that Joyce was not worth bothering about.

One should approach *Finnegan's Wake* through *The Artist as a Young Man and Ulysses*; one should approach *Process and Reality* through *The Principles of Natural Knowledge, Symbolism, its Meaning and Effect* and others of Whitehead's earlier writings, including *Science and the Modern World* (recognising that, however, as being something of a popularisation). Although I took this course in both cases, I confess I never quite made the grade to a confident mastery of either of the two *magna opera*—both a bit too big for their boots. I will go along with Atkinson that he is a difficult author, but that is more because his ideas are unfashionable and profound than on account of his readiness to redefine words to suit his own purpose.

Whitehead totally inoculated me against the present epidemic intellectual disease, which causes people to argue that the reality of anything is proportional to the precision with which it can be defined in molecular or atomic terms. Whitehead's main thoughts were *before* quantum mechanics; his refusal to grant prior validity

to matter or atoms was not so much because no one knows what they are, but rather because all our knowledge has its roots in experience. Science is not based on physical or material realities, it is based on experiments. An experience is something more immediate than the distinction we are immediately tempted to make within it, into the objective and the subjective; it contains both of them.

Whitehead's major thesis was the rejection of what he called "The Bifurcation of Nature", into Mind and Matter, set against one another as two totally separable and incomparable essences. Of course it is often convenient to work with a rough and ready distinction for particular purposes. But he was—and I am—totally opposed to an unbridgeable gap between them—the "Cartesian Dualism", the "Naturalistic Fallacy", and all the rest of the rigmarole which is bringing science into such disrepute with the open-minded younger generation of today. If I had started reading philosophy a few years later, Whitehead would already have been pushed out of fashion by Wittgenstein and Russell, and I would probably have failed to grasp this point. □

Festschrift



Arthur Koestler. Photo: Michael Brett

Astride the Two Cultures: Arthur Koestler at 70. Edited by Harold Harris. Pp. 219. (Hutchinson: London, 1975.) £5.50.

I do not see how it can help but look singularly graceless to take up a critical stance towards *Astride The Two Cultures*, a *Festschrift* for Arthur Koestler on his seventieth birthday. Graceless or not, I have to disclose immediate disappointment at not finding a contribution originating in what I nowadays call the hard-core sciences, those grouped round mathematics and physics.

When C. P. Snow coined the phrase "The Two Cultures", it was clearly the hard-core sciences which he saw at the one pole; and Mr Koestler in his

own writings handles some of the concepts of theoretical physics. The contribution coming nearest is by W. H. Thorpe, the distinguished animal ethologist: he deals with Mr Koestler's views on reductionism and with his ideas about the hierarchical nature of 'living organisation', making use in his frame of reference of the philosophies of Karl Popper and Jacques Monod—good hard-core thinking. Naturally Professor Thorpe is not the only writer to notice the parallel between Mr Koestler's ideas about hierarchy and General Systems Theory. (Briefly: Mr Koestler remarks that an element of a living organisation seen from one point of view appears as a non-autonomous part of the whole, but from another point of view as an autonomous individual.) As a literary metaphor it's nice, and falls in with non-deterministic principles, but it suffers from the limitations of literary metaphor. Theoretical physics may be called a mathematical metaphor: the difference is that mathematical metaphor enables you to penetrate to deeper levels of physical understanding, whereas literary metaphor only enhances your view on one level. As with Bohr's statement of Complementarity—it's nice, the more you look around you the more examples you see; but how much further forward does it get you? Thus, but more so, with Mr Koestler's "Janus-faced holons".

Under the current English dispensation you're expected to address yourself, in contributing to a *Festschrift*, to the work of the person being celebrated: under the original German dispensation you get away with printing large slabs of your own work—three of the present social scientist contributors operate on original German lines. One improves the shining hour with polemic against one of his academic colleagues—yes, Academe is located in the provinces. Another provides a large slab about personality traits: I'm sure diagnosing and assessing personality traits finds honest work for psychologists; it's bad luck that the upshot happens to be lifeless. A third studies animal behaviour: often I strongly deplore this kind of study, because it carries a *sub rosa* message of excuse for us human beings doing what the animals do. In this contribution the animal is a species of monkey whose males impress each other by exhibiting their phalli: if there's the *sub rosa* message here, it would seem to me harmless, in fact favourable to the spread of general merriment.

Atop the other pole—the idiom of the title is catching—sits Goronwy Rees with an excellent appraisal of *Darkness At Noon*. I've never been able to understand why first becoming a Communist and then becoming an

anti-Communist should attract a special kind of moral credit such as is not accorded to someone who has had the sense to do neither. Performing two intellectual U-turns may purify a man's spiritual nature, but it doesn't establish him in my opinion as a reliable thinker. What it may do, it seems, is provide him with material for art. Generally speaking the question "Does the end justify the means?" is most suited to a College Debating Society, the answer being given by a count of Ayes and Noes. Mr Koestler, as Mr Rees demonstrates, has transformed an examination of the question into a work of art. I should have thought Mr Koestler's argument was skewed intellectually by the pressure of abnormally strong feelings of guilt—you really can't get to the truth when there's so much guilt about the place—yet as a work of art the book is clearly a classic. But I do feel bound to look back again to the opposite pole, where I find it hard to go along with the claims made for Mr Koestler there. He is extremely clever and he knows what some of the concepts of the hard-core sciences are about: I take leave to question very seriously the uses to which he puts them.

Of recent years Mr Koestler has become increasingly preoccupied with the so-called 'para-' subjects—telepathy, telekinesis, parapsychology and so on. In writing for *Nature* about Aldous Huxley, who also came to have this preoccupation, I lumped them all together under the title 'parascience', where 'para-' according to the Oxford English Dictionary meant 'beyond, wrong, irregular' and according to me could be replaced by 'non-'—'non-' being at only one stage before 'anti-'. In *The Roots of Coincidence* Mr Koestler begins by trying to establish parascience on the same footing as science; by saying that theoretical physics breaks previously sacrosanct 'laws of nature' in for instance the concepts of negative mass and time reversal, while parapsychological research has become more rigorous, statistical, computerised. A specious argument: on the one hand negative mass and time reversal are straightforwardly within the logic of the established mathematical metaphor; whereas everybody knows you can be rigorous, statistical, computerised from premisses that are entirely fallacious. Mr Koestler then goes on to postulate a world of psychic reality alternative to that of physical reality (and interwoven with it in the matrix of 'oceanic reality'; complementarity is invoked, of course), where the seemingly acasual events of the paraworld—amazing coincidences, peculiar premonitions, levitating tables and crumpling spoons—may be as accounted-for as the

casual events of the physical. The world of 'oceanic reality' you reach by way of mystical or mescaline experiences.

One can readily take a magnanimous line towards Mr Koestler as a man. For instance when he flirts in *The Midwife Toad* with Lamarckism, one can see his sense of justice compelling him to establish the claims of dissident off-beat minds in the dialectical process of arriving at scientific truth. And when he attacks determinism one can only respect that element of the human spirit which insists on having some say in its own fate, and which rejects the idea of absolute predetermination and inevitability. But one's attitude to him as a thinker has to be cautious, clever as paint though he may be. One has to watch out for preoccupation with the paranormal turning a man into a parthinker.

William Cooper

Chromatographic

Extraction Chromatography. (Journal of Chromatography Library, Volume 2.) Edited by T. Braun and G. Gherisini. Pp. xvii+566. (Elsevier Scientific: Amsterdam, London and New York, 1975.) Dfl. 130; \$54.25.

THE title of this book will be unfamiliar to most western practitioners of chromatographic separation methods. It is defined in chapter 1 as the reversed-phase partition chromatographic separation of ions usually, although not necessarily, using systems which greatly favour partition into the stationary phase. Various means, including complexing and chelating agents as well as physical partition, are used to achieve this end. The separations described in this book are exclusively inorganic, although this does not seem to be intrinsic in the method.

I personally feel that this nomenclature is unfortunate in that it does not give due emphasis to the distribution between two phases, which is the essential mechanism of all chromatographic processes. It has arisen, however, from the extensive use of liquid-liquid extraction in the separation of the products of nuclear fission, the chromatographic applications having followed later. In so far as the term extraction chromatography is restricted to those inorganic chromatographic separations, in which an ion is converted into a non-ionic complex which is extracted preferentially into a non-aqueous stationary phase; it is probably an acceptable innovation.

Within these limits this collaborative work succeeds admirably. After introductory chapters on the theoretical aspects and the correlation between chromatographic and liquid-liquid partition results, three excellent chap-

ters describe experimental methods (which are necessarily rather elaborate in all reversed-phase partition chromatographic techniques), stationary phases (in considerable detail) and support phases. The last by Katykhin is outstanding and will be very valuable to organic chemists and biochemists as well as those concerned exclusively with inorganic separations. This chapter contains a wealth of tabulated material on all the commonly used support phases, including physical properties such as density, surface area, total porosity, average pore diameter, capacity for typical organic stationary phases and HETP values for a wide range of chromatographic systems. International suppliers, including those in east European countries, are also tabulated. To my knowledge no such extensive compilation has appeared elsewhere. Katykhin also discusses the pretreatment of the support materials and column packing. He also includes a description of the preparation of sintered porous PTFE blocks which can be moulded into any required shape, and can be used repeatedly in a variety of reversed-phase chromatographic systems, including annular blocks for continuous chromatography.

The remainder of the book deals with special applications of extraction chromatography to specific inorganic ionic groups. Stronski deals with the separation of radionuclides, and shows that in the majority of cases a difference of unity in atomic number suffices for complete separation. Separations of the individual elements are also usually possible in most vertical columns of the periodic table, for example, Li, Na, K, Rb and Cs; Ca, Sr and Ba; Au, Pt and Pd; and the halides, F, Cl, Br and I. Complete separations in the IVth, Vth and VIth groups, however, are still difficult. Separations of the actinides and lanthanide elements are described in chapters by Muller and Siekierski.

In summary it seems that good separations of most pairs of inorganic ions can now be achieved simply and effectively by extraction chromatography both on the analytical and preparative scales. The method is particularly suitable for highly radioactive elements, as preparative separations can readily be carried out by simple remote control in well-shielded enclosures, whereas analytical separations can be carried out at such high dilutions that special precautions are usually unnecessary.

This book provides an excellent and complete coverage of the whole field of the separation of inorganic ions by column, thin-layer and paper chromatographic methods, and as such it is highly recommended.

C. J. O. R. Morris

Insect miscellany

Insects, Science and Society. (Proceedings of a Symposium held at Cornell University, Ithaca, New York, October 14–15, 1974.) Edited by David Pimentel. (Academic: New York and London, July 1975.) \$15; £7.20.

THE symposium on which this book is based was held in Cornell University in October 1974 to celebrate the centenary of the teaching of entomology at Cornell—indeed, in the United States—coupled with the name of the founding father John Henry Comstock, who gave the first course of lectures while still an undergraduate. The well known achievements of Comstock are briefly reviewed by E. H. Smith. D. Pimentel gives a general introduction on insects and crop production in the US, and J. J. McKelvey a colourful picture of 'insects and man'. E. O. Wilson contributes a brilliant short essay on the evolution of insect societies, in which their potential for studying the nature of sociality is stressed.

R. D. Alexander gives a lengthy account of chorusing behaviour in Orthoptera and cicadas, which is largely a re-interpretation of the author's earlier observations, the stress now being laid on the selective advantage secured by individual males in the competitive search for a mate, rather than on the advantage for the group. W. L. Roelofs reviews his own researches on the red-banded tortrix (leafroller) as an example of the physiological properties of pheromones and of their potential use in monitoring populations and perhaps in control by trapping or mating disruption. In a stimulating essay J. S. Kennedy argues persuasively for the view that all migration (and that includes 'dispersal') in insects involves a behavioural response, an essential component of which is the suppression of reproductive ('vegetative') activity and its temporary substitution by active locomotion, with or without passive transport by the elements. M. D. Pathak reviews the methods and results of plant breeding for resistance to insect pests as illustrated by the wide ranging studies on rice.

According to a valuable contribution by T. R. E. Southwood the competing models of population dynamics at varying densities all contain elements of the truth, and that an approximation to real life can be obtained by integrating these models into a single framework which also takes account of habitat stability. As P. S. Messenger points out in a paper on parasites, predators and population dynamics in California, that

even in highly developed monocultures the complex of insect species is very large and general predators are a major element in integrated control schemes. W. Klassen gives an account of the planning and administration of the insect control programmes as organised by the US Department of Agriculture, in which it is claimed that increasing stress is being placed on 'integrated control'.

The final article, by L. D. Newsom, deals with 'pest management' (synonymous with integrated control)—the standard procedure of the entomologists of the nineteenth century, which he illustrates vividly in relation to cotton and soya bean culture in the US. There seems little doubt that the future lies with these ecological procedures. But they require skilled and imaginative entomologists; they are vigorously opposed by the salesmen of the chemical industry whose approach appeals more to the grower; and they still lack administrative encouragement. They do, however, have some enthusiasts on their side.

Although not closely integrated, and although reproduced in offset typescript, this book contains a high proportion of valuable articles.

V. B. Wigglesworth

Stellar essays

Problems in Stellar Atmospheres and Envelopes. Edited by B. Baschek, W. H. Kegel and G. Traving. Pp. xix+375. (Springer: Berlin and New York, 1974.) DM48; \$20.70.

THE occasion of the seventieth birthday of one of the world's most distinguished authorities on the theory of stellar atmospheres and the quantitative analysis of spectral lines—Professor Albrecht Unsöld of Kiel University—has been marked by the production of this *Festschrift* to which eleven of his former pupils have contributed review articles on stellar atmospheres and related topics, expressing at the same time the hope that others will find them interesting and stimulating.

This hope is certainly fulfilled by the great majority of the articles, in which the authors give their personal viewpoint on several topics of current interest in stellar astrophysics. Classical problems relating to the structure of stellar atmospheres are discussed by D. Labs, who gives a convincing defence of the solar irradiance tables published by Labs and Neckel in 1968 and 1970, and by Mrs E. Böhm-Vitense

who uses a grid of model stellar atmospheres in radiative and local thermodynamic equilibrium to predict continuous spectra and colour indices as a function of temperature, luminosity and chemical composition. From a less classical point of view, G. Traving discusses the formation of spectral lines in turbulent media and the physical significance of the controversial empirical parameter known as 'micro-turbulence'. Moving out from 'atmospheres' to 'envelopes', W. H. Kegel describes the theory of maser action in OH and H₂O emission sources, while L. Oster gives a rather entertaining historical review of radio emission from stars and its significance—a subject that has been close to Unsöld's heart since the 1950s—and K. H. Böhm describes physical conditions in the rather mysterious Herbig-Haro objects and in the related nebulae surrounding T Tauri stars, which may result from shock-heating by the impact of a stellar wind on the intracloud medium.

The remaining articles concern the application of stellar atmosphere theory to abundances of elements and other problems in stellar internal structure and evolution. K. Hunger discusses the helium stars, concluding that some of the intermediate variety (as well as the extreme ones) achieve this state by nuclear evolution, whereas others may be affected by atmospheric diffusion. Further abundance anomalies in hot stars (mainly with weak helium lines) are lucidly described by B. Baschek, together with the many mechanisms that have been proposed to account for them, among which the diffusion hypothesis of G. Michaud is generally the most successful, although other mechanisms may also be involved. Diffusion is also clearly important in white dwarfs, which are discussed in a useful review by V. Weidemann that covers their role in galactic evolution as well as their atmospheric properties. K. Kodaira describes the spectral analysis of A-type horizontal-branch stars which is of interest both as a source of information on the chemical composition of old objects and as a means of determining the mass-luminosity ratio, which can then be compared with predictions from evolution and pulsation theories; and D. Reimers discusses the rate of mass loss from red giants on the basis of circumstellar absorption-line observations, deriving an empirical formula which shows that observed mass-loss rates are sufficient to satisfy evolutionary requirements for stars having less than twice the mass of the Sun.

Altogether this book is a worthy tribute to its illustrious dedicatee and a welcome addition to the astronomical review literature.

Bernard Pagel

Imaginative reflections

The Lives of a Cell: Notes of a Biology Watcher. By Lewis Thomas. Pp. 153. (Viking: New York; Garnstone; London, November 1975.) \$6.95; £3.50.

FOR once the publisher's blurb is correct; this is an extraordinary book. At first glance this is not apparent. The book is a collection of essays, 29 of them, each no more than about five pages long, reprinted from issues of the *New England Journal of Medicine* from 1971 to 1973. The author is a distinguished clinician, but the essays are not about medicine (except incidentally); most of them are imaginative reflections about societies of insects and men. The best way to convey the flavour of Thomas' book is to say that he looks with wonder and understanding at the behaviour of some simple organisms—in which he includes mitochondria, chloroplasts, bacteria, ants and termites—and his wonder leads him to speculate about the behaviour of man. This theme could be a recipe for sentimental teleology—a sort of latter-day Maeterlinck. But Dr Thomas is too good a biologist to fall into that trap. He knows that to impute even the rudiments of human intelligence to insect communities is nonsense; social insects have no choice but to build nests or hives, to store food, to cultivate fungi, to live in symbiosis with other creatures; all under inflexible genetic compulsion. But (he asks) are there not also examples of genetically coded behaviour in men and communities of men? And it is his speculations about this question which make this book such delightful reading. I use the word 'delightful' deliberately; for the book contains few facts which are not familiar to biologists, and it does not weave these facts into logic, it spins speculations out of them. The essays are little flights of imagination, written with charm and wit. But they are anything but trivial. In practically every essay something familiar is suddenly put into a fresh light, and it looks quite different; and this, of course, is precisely how creative science is achieved. Dr Thomas has a flair for 'thinking otherwise' about familiar things.

For example, he includes mitochondria as 'organisms', for one of his themes is the prevalence of symbiosis in the whole spectrum of nature from termites to men. Mitochondria, he explains, "replicate in their own fashion, privately, with their own DNA and RNA quite different from ours." They are "stable and responsible lodgers" in the human body. Symbiosis is a symbol

of the interdependence of living things, part of the process which constructed metazoans from single cells, communities from metazoans, and ecosystems from communities. He calls as witness the symbiotic service which the protozoan *Myxotricha paradoxa* gives to the termite community, providing the enzyme to break down cellulose.

Another theme of these essays starts with the question asked two generations ago by W. M. Wheeler: "To what extent can insect communities be regarded as organisms?" (It was Hegel who asked a homologous question about the State.) A familiar enough idea, but Dr Thomas illuminates it from a fresh direction:

"Viewed from a suitable height, the aggregating clusters of medical scientists in the bright sunlight . . . at Atlantic City, swarmed there from everywhere for the annual meetings, have the look of assemblages of social insects. There is the same vibrating, ionic movement . . . the darting back and forth of jerky individuals to touch antennae and exchange small bits of information . . ."

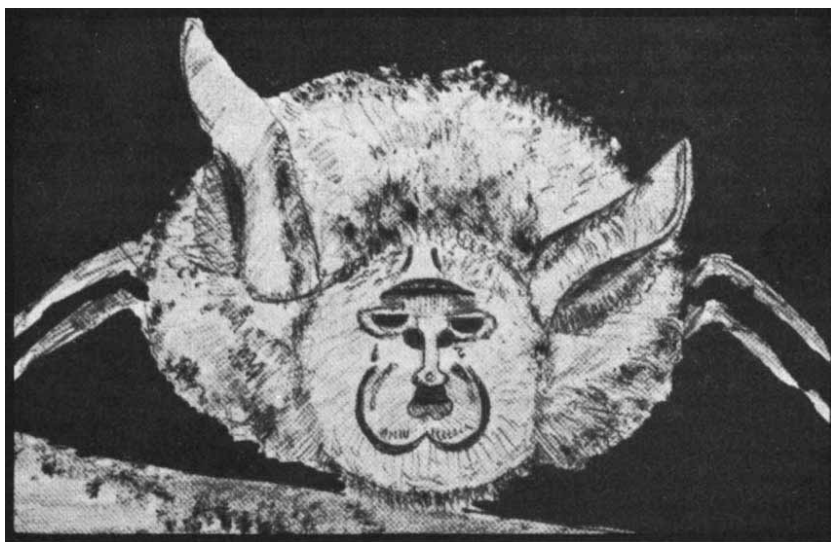
And what are the scientists doing? They "are linked in circuits for the storage, processing, and retrieval of information." What societies of termites do with cellulose, societies of men do with information, rearranging it against randomness into such patterns as quantum mechanics, immunology, genetics. And so Dr Thomas sees an analogy between the termite team (ineffective unless there is a quorum of termites), building something beyond the conception of any of the individual

builders, and the invisible college of scientists, piecing together fragments of information into ordered patterns of understanding. There even seems to a similar compulsion:

"Scientists at work have the look of creatures following genetic instructions; they seem to be under the influence of a deeply placed human instinct. They are, despite their efforts at dignity, rather like young animals engaged in savage play."

As a solution appears they become agitated, as bees do in a disturbed hive, and there "suddenly emerges, with the purity of a slow phrase of music, a single new piece of truth about nature."

Fanciful? Yes, it is; but it is this sort of fancy which acts as a catalyst for hard science. As A. N. Whitehead wrote: "It is more important that a proposition be interesting than that it be true." And Dr Thomas' theme is intensely interesting, for he goes on to suggest that there is one activity of human communities which has an uncanny resemblance to the genetically coded activities of social insects, namely the development and evolution of language. Chomsky has postulated that language must be a biological property of the human mind. The capacity to apprehend syntax is genetically coded. Language grows, changes, evolves, independently of any control or planning by individuals or committees or governments. All of us unconsciously and inevitably contribute to its growth and metamorphosis, with as little understanding of how we do it as a termite has of the architecture of its hill. Language, Dr Thomas says, is "the most compulsively collective, genetically programmed, species-specific, and autonomic of all the things we do, and



Greater Horseshoe Bat with minute eyes on either side of the nose. The curved portion below the nose and surrounding it represents the nose-leaf, which directs sound waves emitted through the nose during flight. Taken from *Vision in the Animal World* by R. H. Smythe. (Macmillan: London and Basingstoke, 1975.) £6.95.

we are infallible at it." Thus, although an anthropomorphic view of termites is undoubtedly bad science, a termitomorphic view of some of man's activities may be illuminating.

There are many other refreshing comparisons between human and animal behaviour in these essays. For instance, a great deal is known about olfactory sensing among animals and insects; but smells play no part in recognition or signalling between human beings. Because of this deficiency (writes Dr Thomas) "we feel somehow inferior and left out of things." Perhaps this sense has atrophied among humans; but the specific smells are still there, for a tracker dog can follow the trail of a man across the tracks of dozens of other persons. A promising line of research here (writes Dr Thomas) for an Institute of Human Fragrance; for if smells are so specific that dogs can distinguish them, have they any link with the equally specific immunological characters in man?

Readers of *Nature*, accustomed to the severe austerity of prose in which a scientific communication has to be made if it is to be credible, may well ask what relevance these whimsical essays have for them. I think they have considerable relevance, and this is why I think so. In the 1970s pure scientific research is on the defensive. Not only is there the first anti-science movement for over a century; there is also, and more ominous, an air of scepticism about the value of pure science on the part of the Establishment. Research Councils are considering the cost-benefit values of their support for some lines of work. Government-sponsored science has now to be regarded as a sort of trade-relationship between 'customers' (who often do not know how to ask the right questions) and 'contractors' (who are paid to answer the questions even if they are not the right ones). In these circumstances it is essential that the public should understand the value to civilisation of encouraging sheer exuberant curiosity about nature. They will not understand this unless they understand what the scientific attitude to nature is like. The end results of scientific research are well popularised in books, films, and TV programmes; but the public know very little about the conception of scientific ideas, the leap of imagination necessary to ask the seminal questions to which research provides the answers. The future patronage of science by the public may therefore depend on a fresh kind of popularisation. If I had to recommend to a non-scientist a book which gives a vivid impression of how scientific ideas are born, this is the book I would recommend.

Eric Ashby

Problems and progress in theoretical physics

Quantum Gravity: An Oxford Symposium. Edited by C. J. Isham, R. Penrose and D. W. Sciama. Pp. 605. (Clarendon: Oxford; Oxford University: London, July 1975.) £10.50.

THE first quarter of this century witnessed two major revolutions in fundamental theoretical physics: quantum theory and relativity. As we enter the last quarter of the century, these two cornerstones of modern physical science are still irreconciled.

Quantum theory was originally developed as a theory of matter, whereas relativity is a description of the structure of space-time and its relationship to gravitation. The latter development of the quantum theory of fields, however, with its spectacular successes in describing electrodynamic phenomena, naturally prompted investigation to determine whether these successes could be repeated for the gravitational field. The only theory of gravity so far tried for quantisation in any seriousness is Einstein's general relativity (and minor modifications thereto), an enterprise which has encountered extraordinarily complicated technical problems, demanding great dedication and courage from would-be quantisers. These difficulties, together with some fundamental problems of principle, have resulted in a whole range of well-tried approaches, all of which have so far failed to yield a satisfactory quantum theory of gravity. Many practitioners, disillusioned with the slow progress of these "frontal assaults", have advocated nothing less than a rejection of the direct use of either general relativity or quantum theory, and propose instead intriguing new principles at a more elementary level of nature. In direct contrast, another popular "second-best" effort has been to try to first cope with the quantum theory of better understood fields which are located in the curved space-time of general relativity, that is, quantum matter in classical gravitational fields.

A whole range of such philosophies and techniques appear in this impressive book. The text, apart from an article by S. Deser, is based on a symposium given at the Rutherford Laboratory in February 1974. There are nine chapters, and although each is by a different author, the organisation and level of presentation are sufficiently consistent to make the book a very useful collection of topics.

The symposium itself was a memorable occasion, because it simultaneously provided a good deal of pessimistic prognosis about achieving a

proper quantum gravity using the more traditional techniques, together with the first public announcement of an extraordinary new result about black holes by Stephen Hawking. There is no doubt that this development, which predicts that black holes create particles and "evaporate" away, has provided a great stimulus for research into quantum field theory in curved space.

Hawking's explanatory article is accompanied by one of the most concise and elegant accounts so far of the theory of twistors, presented in two chapters by Roger Penrose and his former student, George Sparling. Penrose's twistor formalism provides by far the most developed and promising possibility of a completely new approach to the structure of space-time and matter.

The more traditional techniques are dealt with by M. J. Duff, with a clear account of covariant quantisation, S. Deser discussing recent Feynman graph calculations and A. Salam, who outlines some results from his long programme of treating gravity from a particle physicist's viewpoint. The main conclusion to emerge from these discussions is the impasse of non-renormalisability; when matter is present, the divergences which always occur in quantum field theory cannot be isolated for the gravitational field in a systematic fashion.

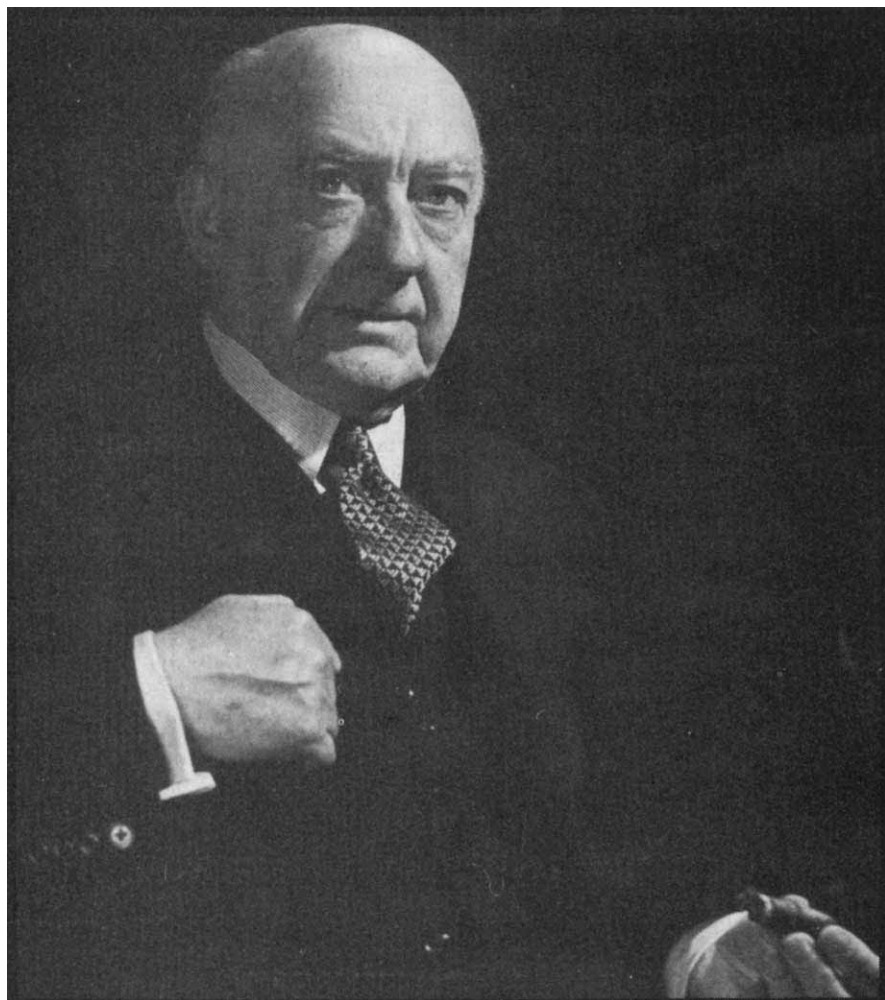
The book also contains a well-written account of some aspects of quantum cosmology—the slightly bizarre technique of quantising the whole universe as a simple mechanical system—by M. A. H. MacCallum. The final chapter carries the unmistakable stamp of J. A. Wheeler in a wide-ranging assessment of the subject, complete with philosophical perspectives and intriguing illustrations. This chapter, entitled "Is Physics Legislated by Cosmogony?", is jointly written by Wheeler and his student, C. M. Patton.

No book of this type could be complete without a good review of the whole subject. A useful and very readable account is given at the beginning by an acknowledged quantum gravity expositor and contributor, C. J. Isham.

For the first time the researcher has available in a single volume a broad range of material, edited by three experts, in this most challenging and difficult area of theoretical physics. It is to be recommended to all departments with an interest in cosmology and astrophysics, and more generally to anyone concerned about the really fundamental aspects of physics.

P. C. W. Davies

Portrait of an industry



Baron McGowan of Ardeer, Courtesy Douglas Glass

Imperial Chemical Industries: A History. By W. J. Reader. Vol. 1: *The Forerunners, 1870–1926*. Pp. xvi+563+38 plates. £7.50 net. Vol. 2: *The First Quarter-Century, 1926–1952*. Pp. xvii+569+41 plates. £18.50. (Oxford University Press: London and New York, 1970 and 1975.)

IN the first volume of this work Dr Reader traced the histories of the four major companies—Brunner Mond, Nobel Industries, United Alkali, and British Dyestuffs Corporation—which in 1926 participated in the biggest merger in Britain, up to that time, to form Imperial Chemical Industries. He gave convincing evidence of his ability to digest an immense amount of detailed information and to present it, very fully annotated, in a readable form. Reader's treatise is, however, essentially a business history—with its emphasis on finance, commercial strategy, and trade practices—and the reader with an interest in the history of technology is less adequately catered for, though he will find the cited references helpful. He will learn more about the consequences of technological

advances than about their causes and natures. There is, for example, rather little about the basic problems of high-pressure technology—fundamental to ammonia synthesis—and how they were overcome. Nor do we learn much of the technical problems of the switch from coal to oil as a raw material after the war. In a history of a pre-eminently science-based industry, where decisions are determined as much by technological feasibility as by commercial acumen, that is a little disappointing.

In the latest volume Dr Reader expounds his main theme, the evolution of ICI during the first 25 years of its existence. As before, he has had unrestricted access to the company's archives and he has written very frankly of failure as well as success, of the weaknesses as well as the strengths of the individuals concerned. As volume 1 relates, the immediate reason for the formation of ICI was McGowan's clear recognition that the existing units of the British chemical industry were too small to compete effectively with the powerful IG Farbenindustrie formed in November 1925. The merger was very hastily cobbled

together—literally as Brunner Mond were on the point of allying themselves formally with IG—and in the early years much effort had necessarily to be expended simply on welding four very disparate entities into a unity which some of them did not really want.

Although a distinguished board was appointed, power from the outset lay with Sir Alfred Mond (later Lord Melchett) and Harry McGowan. After the former's death in 1930 the board elected McGowan chairman and managing director. This unwise dual appointment gave him extraordinary power, of which he availed himself to the full: from 1930 onwards he ruled ICI absolutely. Not until 1937, when he was away in Australia, was his power challenged by the board. Then, just as it seemed that success was within their grasp, what Reader calls "The Barons' Revolt" fizzled out—possibly because of the illness, at the crucial moment, of the second Lord Melchett.

In the early years, ICI's commercial policy was wholly unadventurous. McGowan was a firm believer in limiting production, sharing markets, and—above all—securing to ICI the market in the Empire which its name embodied. To him and his colleagues the Empire was enduring and virtually indestructible: over the years it would provide a reliable and growing market from which all foreign rivals could be excluded, if necessary by making agreements not to enter their territories. It is very reminiscent of Pope Alexander's Bull of 1493 dividing the New World between Spain and Portugal. The idea of competition was positively repugnant. As late as 1941, McGowan wrote that he did not accept "the theory . . . that competition is essential to efficiency". The policy was indeed not unsuccessful at that time but it led to some very serious complications, as, for example, in the case of the Dupont agreement. The United States had very different views of the nature of free enterprise.

It was not the only error. Another was in the great fertiliser complex built at Billingham. Technologically, it was a great achievement, but it proved a commercial disaster when markets could be found for only a fraction of its capacity. Reader brings out one reason for that, other than the slump, very clearly: marketing was defective because ICI failed to recognise the crucial importance of the men concerned and accord them the necessary status. Production methods were good, but it seemed that selling the products—especially to the public as opposed to other manufacturers—was too ungentlemanly to be taken seriously.

The half-hearted approach to profit-

ability was not without national advantage, even if not to the shareholder. There is no doubt whatever, that, over the period here dealt with, ICI was actuated by a high sense of public responsibility. At no time was this better expressed than during the war when the company's resources, not only in production but in very able manpower, was put at the nation's disposal to an extent far beyond the call of duty, with profit as a secondary consideration.

Failures there may have been, but there were resounding successes too, among them polythene and Terylene. Apart from such highlights, however, the substance of the tale told here is one of solid, if unspectacular, achievement over a vast industrial field covering non-ferrous metals, pharmaceuticals, dyes, paints, fertilisers, plastics, fibres, and many other products.

There is one field of ICI's activities deserving greater mention than is given by Reader, and that is the field of publicity and public relations. The company, under the late Sidney Rogerson, pursued an enlightened policy which set new standards. Works were commissioned not only from established artists but also from younger aspirants yet to make their name. Equally high standards were set for the literary style. Older readers will recall such outstanding series of advertisements as 'Ancestors of an Industry', 'Equipment of an Industry', and 'Portraits of an Industry' (from the last of which, incidentally, eight of the illustrations of the book are taken, though the source is not mentioned). Another series, the 'Colours of Chivalry', was ultimately made available as a book which is now a collectors' piece. No mention is made either of *Endeavour*, an international scientific review in several languages, which was started during the war and afterwards established itself as an accepted part of the world's scientific literature. Equally, no reason of modesty should have prevented much fuller mention of the company's enlightened policy towards the universities.

The Postdoctoral Fellowship Scheme, begun in 1944 and only just terminating, gave a start in professional life to well over a thousand able young scientists. Many of them have gone on to outstanding careers in science, and they include two Nobel Laureates.

It is, of course, very easy to criticise a work of this kind on points of detail. Unquestionably, however, this is a masterly account of the formative years of a vast supranational company. Others might have done it rather differently, but it would be difficult indeed to have done it better.

T. I. Williams

Comprehensive biochemistry

Medizinische Biochemie: Lehrbuch für Studierende und Ärzte. 6., Völlig neu bearbeitete Auflage. By S. M. Rapoport. Pp. xxvii+1023+14 tafeln. (Volk und Gesundheit: Berlin, Jena 1975.) DM53.

Comprehensive Biochemistry. Vol. 31: A History of Biochemistry. Part 3: History of the Identification of the Sources of Free Energy in Organisms. By Marcel Florkin. Pp. xx+475 (plates 64-120). (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.130; \$54.25.

Of its kind Rapoport's is one of the best German textbooks of biochemistry for medical students. The book is too detailed for mere preparation for preclinical examinations but is very suitable for keen students, especially those in their clinical years and beyond, who wish to read about the biochemical background of diseases. The material is well selected with special reference to the practice of medicine. The fact that the book has reached its sixth edition since it was first published in 1962 is a measure of its popularity. The new edition is brought up to date with the help of several close collaborators.

What makes the book attractive is its coherence and readability. This is achieved by linking general comments, often of a highly original character, and by avoiding the mere assembly of factual information. The interest of the medical student and medical graduate is maintained by continually relating the biochemical information to normal and pathological organ function. Selected references for advanced reading are given. More frequently than is usual in western treatises are references to Russian investigators and to the importance of their contributions to the progress of the subject.

The text is occasionally interspersed with references to Marxist philosophy—for example, by quoting (on page 35) Engels' formulation of the nature of life: "Life is the manner of existence of protein, the chief property of protein being the continuous exchanges of material with the external environment the cessation of these exchanges causes the decomposition of proteins". This statement is taken to relate to the dynamic state of proteins, predicted in this passage by Engels many decades before it was experimentally established.

FLORKIN's work is the first comprehensive effort since Lieben's *Geschichte der Physiologischen Chemie* (1935) covering a field which has grown to vast dimensions in recent decades.

sions in recent decades.

This is the second of four volumes planned as the last section of the 33 volumes of the reference work *Comprehensive Biochemistry*, edited by Marcel Florkin and Elmer Stotz. Florkin, who is writing all the four volumes, is well qualified to undertake this difficult task as he is a biochemist who has researched in many areas, especially in comparative biochemistry and has had the unusual distinction of having been president of the International Union of Biochemistry—an indication of his standing as a biochemist, president of the International Academy for the History of Medicine and vice-president of the International Academy of the History of Science, on account of several major publications on historical aspects of medicine and science.

The present volume is concerned with the history of the sources of energy in living organisms, that is, fermentations, glycolysis, biological oxidations and photosynthesis. The narrative is detailed, giving the key references to the original papers, quoting literally many telling passages, and reporting the story of the complex ways by which the edifice of knowledge gradually took shape—a process which involved not only construction but a fair amount of demolition. Individual scientific publications often give an impression of a continued series of successful experiments because all the disappointing failures (although they may have been essential and unavoidable steps) have been omitted from the final presentation. At a different level this is also true for the history of scientific developments. Florkin illustrates this by relating many of the aberrations which are part and parcel of the steady progress of science.

Students of science are nowadays often disinclined to concern themselves with the historical development of their subjects. They feel that there is so much to read and to learn about the present state of affairs that there is no spare time for concerning themselves with how the knowledge was arrived at, and in particular with reading about aberrations which led to dead ends. Understandable though this attitude may be, for the proper appreciation of scientific knowledge it is essential, especially for those engaged on research, to know how knowledge was arrived at.

The way in which Florkin writes makes it pleasant and easy to get glimpses of the historical background of present knowledge. I feel confident that the book will be enjoyed by many readers, and as a source-book it will remain a standard reference work for a long time to come.

H. A. Krebs

Common sense, wa hae



The Triumph of Truth by Joshua Reynolds, 1774. The figure on the right portrays James Beattie (1735-1803) holding his *Essay on Truth*. The Angel of Justice is thrusting down the head of Voltaire. The head in the foreground was believed by David Hume to be his; that in the left corner was said to be of Edward Gibbon.

Scottish Philosophy and British Physics, 1750-1880: A Study in the Foundations of the Victorian Scientific Style. By Richard Olson. Pp. vii+349. (Princeton University: Princeton, New Jersey, and London, August 1975.) £9.20.

"WHERE we try to find models or analogies, they are quite content with laws", so wrote H. G. J. Moseley in his diary for June 1914 after meeting the French physicist Urbain. And if "pictorial thinking" has been a major and distinguishing characteristic of British physics, it is tempting to speculate on how it came about. The interesting thesis of Dr Olson is that it started with the 18th century Philosophical Society of Aberdeen (also known as the Wise Club) whose two most influential members were Thomas Reid, Professor of Philosophy at King's College, and James Beattie, Professor of Moral Philosophy at Marischal College.

The Society was the cradle of Scottish Common Sense Philosophy, so little known today that it has no mention in Bertrand Russell's *History of Western Philosophy*. It arose as a reaction against the atheistic scepticism of David Hume (to whom in fact it owed a good deal) and the materialism of thinkers such as Voltaire and Joseph Priestley. The Aberdeen group, in their efforts to defend moderate

Scottish Presbyterianism against the rationalist challenge, had encountered the difficulty that many of us currently face in battling with the prospect of anarchy based on specious rationalism: the latter can lead to conclusions which in Reid's words contradict "certain principles which the constitution of our nature leads us to believe, and which we are under a necessity to take for granted in the common concerns of life". These are moral principles which can be no more proved than the axioms of Euclid and yet which are correspondingly essential as the foundations for a framework of morality.

This seems to be a remote base from which to trace the rise of pictorial thinking in British physics, but it is what Dr Olson sets out to do:

"The basic aim of this book is to present and to establish the probability of a hypothesis regarding the development of the exact sciences—that is, that many of the important characteristics of the exact sciences in Scotland, and subsequently throughout Britain can be accounted for by the fact that Scottish scientists adopted a particular set of methodological and epistemological attitudes which were clearly articulated by a group of moral philosophers collectively known as the Common Sense School".

Perhaps the most suggestive argument is that with its reliance on common sense plus a few moral axioms,

Scottish philosophy had greater faith in the pure "geometry of visibles", proving results by Euclidean arguments, than in analysis, where the algebra of infinite series was known to be dangerous, and where some of the entities such as the square root of minus one had no common-sense physical interpretation. Certainly, there was strong interaction in the Scottish universities between the moral and the natural philosophers (Beattie was in fact appointed to the Chair of Natural Philosophy but exchanged it the same day for that in Moral Philosophy) and undergraduates were exposed to both influences in the traditional Scottish degree.

And there was plenty of good teaching to be had: Reid held with Bacon that the stages of human knowledge were "like steps of a ladder" in which it would be "fruitless to skip directly from bottom to top"—a lesson often ignored today with disastrous results in school teaching. We find John Playfair distinguishing between hypothesis and theory:

"Hypotheses are explanations which have no evidence independent of their ability to account for the phenomena in question while theories are founded on facts known independently of the phenomena to be accounted for".

And William Hamilton extended Occam's Razor into the Principle of Parsimony:

"neither more nor more onerous causes are to be assumed than are necessary to account for the phenomena".

Again, Hamilton spoke of

"the highest faculty of the mind—that of tracing the analogy of unconnected observations of evolving from the multitude of particular facts a common principle, the detection of which might recall them from confusion to system, from incomprehensibility to science".

Hamilton's colleague at Edinburgh, J. D. Forbes (of the bar and glaciers, and the Chair of Natural Philosophy) wrote

"The importance of analogies in science has not, perhaps, been sufficiently insisted upon by writers on the methods of philosophizing. A clear perception of connexion has been by far the most fertile source of discovery".

Dr Olson ascribes the demise of the Scottish common-sense tradition to Forbes because his advocacy of greater specialisation in science at the undergraduate stage dethroned moral philosophy from its paramount place in Scottish education. But he as well as Hamilton influenced their pupil, Clerk Maxwell, who all through his life was fascinated by analogy—for example asking the question in a postgraduate essay "Are there real analogies in nature?", and repeatedly stressing the fruitful function of analogy throughout his papers and addresses. As a

useful to alert potential readers to culmination, his Electromagnetic Theory showed him to be the greatest of all masters of analogical thinking.

This may be the point at which to mention some very minor blemishes in the book, for Maxwell spent his boyhood not in Aberdeenshire (p. 287) but Kirkcudbrightshire. Melloni's Christian name was Macedonio not Marcello (p. 227) and Poynting's initials were J. H. not M. H. (p. 328), and there are occasional misspellings.

Whether through common-sense Philosophy or not, the Scottish contribution to science up to 1870 was formidable. Besides those of Forbes and Maxwell it is easy to recall many names: Black, Brewster, Brown, Graham, Hutton, Leslie, Lyell, Nicol, Rankine, Ritchie, Robison, Tait, Waterston, Watt, the Gregorys, the Playfairs, and the Thomsons. Quite possibly there was more to it than the common-sense tradition alone: in Britain generally, religious dissent also seems to have been a factor, and the relative decline of the Scottish universities after 1870 was probably far less due to Forbes than to the revival of Oxford and Cambridge, with the abolition in 1871 of religious tests for university entrants. But Dr Olson has produced a book of remarkable interest, well worthy of detailed study by everyone interested in the philosophy of science.

R. V. Jones

Earth studies

The Earth's Density. By K. E. Bullen. Pp. xiii+420. (Chapman and Hall: London, 1975.) £12.90.

This book gives an historical account of developments concerning the variation of density within the Earth. The first five chapters are of an introductory nature covering noted ancient investigations, the determination of the Earth's mean density, spherical harmonics, theory of the Earth's gravitational attraction and the figure and moment of inertia of the Earth. The sixth chapter covers early models of the Earth's density variation including the Legendre-Laplace density law and the Williamson-Adams equation. The meat of the book (chapters 7-17) deals with seismic wave transmission and presents contributions from studies of P and S waves, surface waves, and free oscillations. The penultimate chapter deals with optimum and standard Earth models and the final chapter contains a discussion of the densities of the other planets.

The author, a mathematician, has been foremost in developing Earth models of the classical type based on the interpretation of travel time curves, and chapters 9 and 10 provide a resumé of the Bullen Earth Models of types A and B. Other

procedures, such as the Monte Carlo technique and general inversion, are also discussed but somewhat unenthusiastically. The reader will naturally turn to the chapter on optimum and standard Earth models and there he may be disappointed. It seems we still do not have a standard Earth model and the Monte Carlo and general inversion models are treated with scepticism. Bullen's choice of the best model is shown in Fig. 16.1 but it is not clear which model this is and no error bars are shown; it is presumably a combination of Bullen-Haddon models. It is unfortunate that the big conclusion is somewhat obscure as the rest of the book is so beautifully meticulous.

Throughout, the Earth is assumed to be spherically symmetrical, and though lateral variations of density are frequently discussed they are usually dismissed as minor. This is a pity, as with more precise data (using WWSSN and seismic arrays) it is becoming apparent that the Earth is far from spherically symmetrical and that there is considerable local variation in travel time curves. Most of the work described dates from a time before the acceptance of sea floor spreading and 'rapid' plate movements. The outermost layers of the Earth are very mobile and we have few clues as to what causes the mobility, so studies of lateral variations of density are clearly of great importance. We will need to know how big they are and how deep they extend. With that kind of information it may become possible to develop theories to explain plate movements and the earthquakes associated with them. In the meantime, Bullen's book gives us all the hard facts of classical seismology with the warning that they mustn't be forgotten as the lesson for the day.

R. W. Girdler

The Viscosity of the Earth's Mantle. By Lawrence M. Cathles III. Pp. 386. (Princeton University: Princeton and London, 1975.) £13.10.

THE viscosity of the Earth's mantle is obviously important, not least because of the restrictions that its absolute value and variations with depth place on possible models of mantle convection. Nevertheless, it comes as something of a shock to learn that it can form the subject of a complete, long book. The shock is magnified (or perhaps diminished, depending on one's point of view) by the discovery that, although previous literature is briefly reviewed where appropriate, this is not a textbook but an original work. In short, it is really a long scientific paper which happens to be bound between hard covers. The normal rules of book reviewing therefore hardly apply; proper criticism of this work will only come from other workers in the field over a period of years, rather than weeks or months.

In the meantime, it would seem most

Cathles' chief conclusions, especially as one or two of them, if not actually controversial, certainly defy the consensus. The study is based on the assumption that the Earth is a self-gravitating viscoelastic solid; and in the first part of the book the mathematical techniques required to model the isostatic adjustment of such a body are developed at length. The theoretical foundations thus acquired are then combined and compared with geological data on the way the Earth responded to Pleistocene glacial-water load redistributions to deduce mantle viscosity and its variations.

The 'average' Earth model thus constructed has a lithosphere which may be regarded as an elastic shell with effectively infinite viscosity and thickness varying between 70 and 150 km. Immediately below the lithosphere is a low viscosity layer (or 'channel') with an average thickness of about 75 km and a viscosity of about 4×10^{20} poise. The evidence for such a channel is widely distributed geographically, although both the thickness and viscosity of the channel probably vary considerably from place to place. Between depths of 75 and 1,000 km measured from the top of the low viscosity channel, the mantle (defined as the 'upper mantle') has a viscosity of $1.0 \pm 0.1 \times 10^{22}$ poise—a conclusion based largely on the uplift of Fennoscandia which has a further 30–50 m to rise.

Most interest, however, is likely to centre on the rest of the mantle (the 'lower mantle') which also has a viscosity of about 10^{22} poise, although at $0.9 \pm 0.2 \times 10^{22}$ poise it might be very slightly lower than that of the upper mantle. Many (but not all) workers believe that the lower mantle has a much higher viscosity and that mantle flow is limited to the asthenosphere. But a viscosity of about 10^{22} poise throughout permits full-mantle convection. Moreover, if full-mantle convection is occurring, its main function must be to transport heat from the Earth's interior into space (by way of lithospheric conduction), which can be done effectively only if the upwelling convective limbs are thin. In other words, Cathles' analysis supports mantle plumes, whether cylindrical or sheet-like. Following Morgan, the plumes may then be envisaged as spreading out at the top into the low viscosity channel, with at least a part of the convective return flow occurring uniformly through the mantle as a whole.

Last, but not least, Cathles' model assumes right from the start that viscosity is Newtonian throughout the mantle. This turns out to be entirely consistent with glacial uplift data.

Peter J. Smith

Pantheism in modern dress

ALISTER HARDY is not the only distinguished biologist to believe in god, or to attempt the reconciliation of that belief with the findings of modern science. In this book* he touches on many of the common arguments, but makes no excessive claims for them, alone or together. Human societies have commonly adopted religions, however disparate—thus, religious belief may be held to be natural to man. Human moral values have varied, and do vary, but to possess them at all is evidence of divine inspiration. The subjective, but clearly not rare, experience of an exaltation, which does not have an obvious explanation in drugs or other definable agents, is in one sense quite real. Hardy sees them all as simultaneously manifestations of natural law (with their analogues present in infra-human species) and of the divine.

Thus he is prepared to see a dog's adoration of its master as equivalent to man's of his gods. Life in a hunting pack gave selective advantages to the capacity to be a follower. The rituals of worship he finds to be ethologically respectable gestures of submission, and this leads him to invite us not to feel embarrassed at reciting the Lord's prayer on our knees. It is nature's way of expressing devotion. But to what? To nature, seems to be the answer.

Indeed, if I understand Hardy's position correctly, it is not easily distinguishable from pantheism, and this will present both his theist sympathisers and his atheist critics with difficulties. The gods which old-fashioned atheists are accustomed to be without are fairly well defined. Their interventions in our affairs are particularly impressive when they flaunt natural law. As swans or as the holy ghost they impregnate women and tangible offspring are the result. They listen to human prayers and may or may not, as they see fit, save the king, connive at the destruction of Troy, or give us our daily bread. But Hardy's version of natural theology must leave us very much in doubt about what it is we are being invited to accept or provoked to reject.

One difficulty lies in his rather ambivalent approach to "paranormal" phenomena. He discusses extrasensory perception (ESP) telekinesis and apparitions, but rather tentatively. Paradoxically these phenomena are only especially relevant if they do not normally

occur. If ESP and psychokinesis are reproducible phenomena they are on the agenda of science. If they are not, but occur as rare and unpredictable events which might reflect the decisions of a supernatural power, then it is open to believers to use them in support of the hypothesis of god. We cannot forget, however, that our history is littered with phenomena which started as unpredictable, but are now regarded without undue awe—floods, for example.

We may accept that otherwise sane and sober people have 'seen' apparitions, without admitting that these were objectively visible. Hardy does not imply, for example, that a stranger bursting in on one of Canon Phillips' interviews with the ghost of C. S. Lewis would have seen it too. The question

is whether dreams, apparitions, and the like are going to yield entirely to psychological, neurophysiological and psychopharmacological analysis, or are events through which supernatural powers have access to our minds. Hardy seems to want to have it both ways. He accepts scientific analysis and the natural origin of the human faculties he discusses, and he is therefore forced to find the divine permeating nature, but only achieving its full stature with the evolution of man. For this, I suspect, traditional believers will not thank him. A god that is not in some sense independent of nature cannot really compete with Zeus.

Hardy presents his views discursively, modestly, and for my taste in altogether too good-natured a manner. If atheist biologists, such as Monod, are wrong their works should be criticised, not combed for sympathetic quotation. Just a little asperity would have done this book a world of good.

D. R. Newth

Origins of modern science



Collection Allen G. Debus

Robert Fludd (1574–1637). From the *Integrum Morborum Mysterium: sive Medicinae Catholicae Tomi Primi Tractatus Secundus* (Fitzner, Frankfurt, 1631)

The most provocative essay, from the 'mystical' side, is that on Newton's alchemy, by Professor Richard Westfall. Having argued in earlier studies that Newton was not above breaking the rules of scientific method, Westfall describes the enormous bulk and complete technical mastery of the Newtonian alchemical corpus. What else can be said? Newton was clearly committed to chemistry far more than his publications indicate: he was sure that the alchemical tradition contained important secrets; and yet it is highly unlikely that he participated in the mystical aspects of the great work. But was it only chemistry? The matter rests undecided as yet.

A reminder of the importance of the genuinely alchemical and mystical traditions even in the 17th century is given by Alan Dobus. He reviews the career of von Helmont, the mildly Paracelsian chemist, whose sufferings at the hands of the Inquisition are totally neglected in the folk histories of science, perhaps because they were in the cause of a 'prescientific' world view. Also, there is Robert Fludd, the propagandist for a mystical philosophy, who was a close friend of William Harvey, and who defended the circulation of the blood against Gascendi's claims of the existence of the interventricular septum.

Another lively topic canvassed here is the quality of Galileo's reports of his

ALTHOUGH some might be misled by the title of this book† into expecting rather more than is delivered, this is nonetheless a very useful and interesting book. What it does not provide is a serious discussion of the relationships between the three 'themes' of the title. But it does contain useful essays on the separate aspects, as well as a review of the current controversy among historians of the scientific revolution.

**The Biology of God: a Scientist's Study of Man the Religious Animal*. By Alister Hardy. Pp. 238. (Jonathan Cape: London, July 1975. £4.50.

astronomical observations. Paul Feyereabend has accused Galileo of faking these and other reports, and certainly the matter is far from straightforward. Although the experts (Righini and Gingerich) disagree, there seems a consensus that it was more a matter of a combination of genuine difficulties, haste and wilfulness than a deliberate or serious deception. Other Galilean topics include an identification by A. C. Crombie of the textbooks from which Galileo derived his early Aristotelian ideas; and a scholars' debate, perhaps unresolvable, on the interpretation of manuscripts purporting to record early experiments on free fall.

A general view of the problem of this book's title is offered by Professor Rupert Hall and Paolo Rossi. Theirs are polemical pieces directed against the advocates of the 'influence' of the alchemical-mystical tradition on the new science. Certainly, they could not find a more knowledgeable and sympathetic antagonist than Rossi, since his studies on Bacon were among the first to exhibit the interplay of continuity and change in the origins of the new natural philosophy from its roots in traditional philosophies, rhetoric, the mechanical arts, and magic. Yet he has chosen to refute rather than assimilate the new claims.

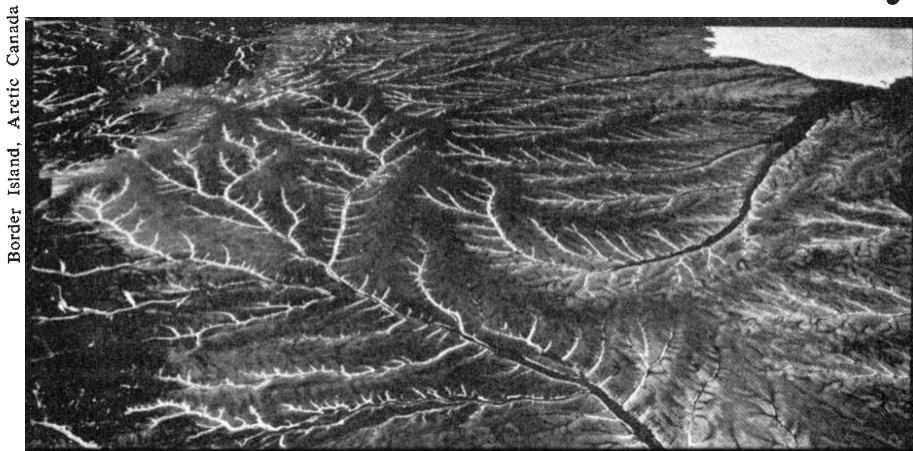
The absence of an ecumenical survey of the problem reflects the fact that at root the debate is ideological. With all that we may now have to admit of the imperfections of the enlightenment and the positivist views of science and its origins, to some it is still a question of rationality in eternal struggle with the irrational. I personally think that Professor Hall is too restrictive, as when he condemns Namierite history as 'irrational' because it ascribes private personal motives for political action. But he and Rossi are together in what seems to be their implicit understanding of the 'irrational', as any view that supposes the faculty of 'rationality' to exist outside the minds of man. By such a criterion much of the hard scientific work on which the scientific revolution was based was accomplished by men who fall wholly or partly under the ban, as Gilbert, Kepler and Harvey. Clearly, the matter needs further analysis.

As a record of a symposium, this is a testimony to the skills of the organisers in bringing together an unusually distinguished and lively gathering. As an exploration of a new facet of a perennial problem, 'the origins of modern science' it represents a good start.

J. R. Ravetz,

†*Reason, Experiment and Mysticism in the Scientific Revolution*. By M. L. Righini Bonelli and William R. Shea. Pp. 320. (Macmillan: London and Basingstoke; Science History: New York, 1975.) £13.00; \$20.00.

Fluvial controversy



The Work of the River: A Critical study of the central aspects of Geomorphogeny. By C. H. Crickmay. Pp. xvi+271. (Macmillan: London and Basingstoke, January 1975.) £12.00 net.

FOR over fifty years the author of this book has been concerned with the study of physical geology in the field and the interpretation of scenery in terms of fluvial processes. Here he summarises his findings and theories in a format suitable for students and indeed for anyone interested in river-shaped landscapes.

First, the reader is introduced in an elementary way to the forms of moving water on land surfaces, to the nature of the seasonal work of rivers and to the physical attributes of riverine environments. There follow more advanced discussions on fluvial dynamics and channel equilibrium relationships, with much on grade and interesting comments on other aspects such as cavitation. The erosive work of rivers is then outlined with special attention to base level and a chapter is devoted to examining the hypotheses that constitute the beliefs of several schools of geomorphology.

How then does the author interpret real scenery or landforms seen in the field today? To him the key lies in the great difference between the rate of erosion in river valleys and on flat areas. His main postulations are the hypothesis of unequal activity and the concepts of activeness and stagnancy in landform development. "Some areas of the Earth's surface are almost immune from the postulated [by others] universal destruction by wasting". Ground that is nearly flat or gently sloping (that is, with slopes of under about 5°) makes up the main part of the land surface of the Earth. This abundance of flat land in patches of all sizes up to vast expanses "demonstrates that most denudation is horizontally pursued" by the lateral swinging of rivers mainly at grade.

Natural scenery consists of an endlessly varied combination of flats and slopes but since absolute horizontality is rare the Earth's surface may be classified into very gentle gradients or flats and steeper gradients or slopes. Their most significant general relationship is the repetition of one above the other vertically. The flats are stagnant, the slopes active. If stagnant, a geomorphic form may persist almost indefinitely. Although rivers are active erosion agents and wasting may break down all steep slopes, there are certain tracts that survive this destruction because they are out of its reach. When the peripheral flatness extends into the central remnants and predominates there, the land has become a true *Endrumpf*. The ultimate product can only be one broad, low level flat.

These general assumptions mean that most existing scenery was made in time long past and is today essentially relic material. Mountains are areas of "activeness", and the author takes the view that "periods of deep diastrophic calm" are longer than epochs, such as the present, which are characterised by diastrophic agitation and frequent uplifts. The inclusion in the arguments of what to many readers will seem the obvious and the controversial is characteristic of the whole theme. But much of the accepted creed is clearly stated and will be of considerable practical value to earth scientists. Controversy seems inevitable in these natural sciences. For example, is continental drift or mountain building ever still? What is the base level of erosion when low tide mark is far below sea level? Would not high tide and waves consume any flat land mass at base level? But this is the sort of controversy the author deliberately sets out to provoke. Students and earth scientists will find his book a stimulating addition to their bibliographies and as beneficial as a dose of vitamin C after a long season of hibernation.

Robert P. Beckinsale

Evolution of algal ultrastructure

THE green algae are now generally accepted as the evolutionary progenitors of land plants and this book* is an attempt to discuss evolutionary trends within green algae on the basis of cell ultrastructure. After a general introduction, six chapters are devoted to a detailed examination of the ultrastructure, cell division and reproduction of an order of the Chlorophyta. Within each order a restricted number of genera are used to illustrate the diversity of cell structure and reproduction. This approach, although suffering from the potential hazard that the genera selected may not be representative of their order, has been necessary because of the lack of sufficient ultrastructure studies of many green algae. The description of each genus is accompanied by numerous light photomicrographs and electron micrographs (both transmission and scanning), many previously unpublished, which are of consistent good quality and well reproduced. The profusion of photographs accompanying some genera has unfortunately resulted in a loss of synchrony between the text and relevant photomicrographs, such that the reader is obliged to constantly refer to figures several pages distant from the text.

The final chapter is devoted to a discussion of possible evolutionary trends within the green algae by comparison of certain ultrastructural characters. It commences with a list of suppositions concerning primitive and advanced characteristics. The unicellular condition, the possession of basal bodies, flagella, a closed centric spindle, a phycoplast and cell division by furrowing, are deemed to be primitive features, whereas it is suggested that evolutionary advancement can be detected by a colonial tendency, a phragmoplast and cytokinesis by way of cell-plate formation. It is on the basis of the presence or absence of these characters that theoretical evolutionary lines are constructed.

The acceptance of a chlamydomonad cell as the ancestral type of all the green algae is disputed and the hypothesis advanced that this type of cell was the progenitor only of phycoplast-containing green algae. It is argued that the archetypal cell of the other green algae was more like that of

Pedinomonas (Prasinophyceae). From this ancestral flagellate unicell there developed the filamentous form along two distinct lines. The siphonous line (that is the coenocytic condition) is seen as an evolutionary *cul-de-sac*, whereas evolution by way of ulotrichalean types is suggested as the line leading to the bryophytes and eventually higher land plants.

The arguments for these proposed evolutionary lines are well presented, although occasionally repetitive, and the importance of ultrastructural evidence for evolutionary trends, and also taxonomic classification, is strongly emphasised. The main criticism of this significant work must surely be its price. This has presumably resulted from the inclusion of almost 800 individual photomicrographs, but must place this book well beyond the financial reach not only of students but also of many individual research workers.

T. W. Ford



A grimace in an adult female rhesus monkey. Taken from *Primate Behavior: Developments in Field and Laboratory Research*, volume 4, edited by Leonard A. Rosenblum. Pp. viii + 407. (Academic: New York and London, July 1975.) \$29.50; £14.15.

Evolutionary sex

THIS is an excellent little book† on a big subject. One would think that a topic of such great interest would by now have been adequately understood. On the contrary, within the past few years the evolutionary reasons for the phenomena of sex have become increasingly anomalous.

A few years ago it was suggested that the very existence of sexual reproduction constituted a paradox.

For if an organism reproduces by meiosis and recombination with a partner, it 'throws away' half of its genes. An organism that reproduced asexually would have twice the number of its genes represented in the next generation than a sexual one. For sexuality to be selected, an organism would have to possess twice the fitness of asexual ones, and this seems too high.

Presumably, stimulated by this apparent paradox, Williams has undertaken a series of conceptual studies that show how and why this does not really follow. Some of his models are quite convincing, and we are relieved to learn that there are a number of situations in which the fitness of the sexual creatures is high enough to enable sex to evolve. He does not, however, manage to come up with a very satisfactory explanation of why so many sexual organisms exist that do not fit the model. The explanation he proposes is historical accident. He argues that the present-day organisms that should not be sexual are descended from ones that should, and the change for some reason was irreversible. This may be so, but perhaps more adequate hypotheses are in order.

In addition to the big problem, William discusses a number of other issues in the evolution of sex and of hermaphroditism, masculinity and femininity, and some issues in group selection. A number of these matters are given only cursory treatment. For instance, sexual selection is not covered, although sexual dimorphism is reproduction. These include aspects touched on. And the topic of hermaphroditism is made to seem more simple than it really is. I would have appreciated a somewhat longer discussion of these peripheral issues, with more empirical support. Indeed, the lack of facts has been a major drawback in the literature. Although Williams has considered the facts seriously, the reader will perhaps feel a need for more data.

If the facts do not accord with theory, one corrects the theory. Williams's book substantially increases the opportunities for both theoretical and empirical research. Perhaps evolutionary biology is going through a stage like that in astronomy when the epicycles began to become an embarrassment. The time may be ripe for a bolder approach to the subject, and for "theories more pleasing to the mind."

Michael T. Ghiselin

**Green Algae: Structure, Reproduction and Evolution in Selected Genera*. By Jeremy D. Pickett-Heaps. Pp. vii + 606. (Sinauer: Sunderland, Massachusetts; Freeman: Reading, July 1975.) £23.40.

†*Sex and Evolution*. By George C. Williams. Pp. x + 201. (Princeton University Press, 1975.) \$13.50.

Pollution of the atmosphere

The Changing Global Environment. Edited by S. Fred Singer. Pp. viii+423. (Reidel: Dordrecht-Holland and Boston, Massachusetts, 1975.)

Cloth Dfl. 95; paper Dfl. 50.

Chemistry of the Atmosphere. By Murray J. McEwan and Leon S. Phillips. Pp. ix+301. (Edward Arnold: London, 1975.) £9.75.

ANYONE curious about the atmosphere and pollution on a global scale will probably enjoy reading these two books. *The Changing Global Environment* is a collection of scientific papers. It gathers together information and ideas of the decade 1963-73 on the possibilities of man-induced adverse changes in the global environment. In the introduction William Kellogg presents a convincing and balanced overview of the possibilities of man-induced climatic change. The authoritative but disparate views of climatology of Murray Mitchell, Reid Bryson and Syukuro Manabe have been expressed elsewhere but they are well worth having side by side in one volume. This is true also of the other sections which concern such problems as ocean pollution and the disturbance of the nitrogen cycle of our planet. Robinson and Robins efficiently summarise and bring up to date their inventory of the sources of atmospheric pollutants and compare them with the outputs of the same compounds from natural sources. The book comforts neither the doomsster nor the advocate of unrestrained industrial growth but most of the papers are thoughtful and thought provoking, and will make this collection valuable for a while as a source book for those concerned with global pollution problems.

Just as the environment is changing rapidly so is our knowledge about it and inevitably such a collection has the frozen quality of a group photograph. The participants of the debate all seem to have been caught in mid-sentence. The slightly unreal air of this book is well captured in the second paragraph of the introduction: 'We are now in a period of extensive glaciation. The previous interval occurred 300-250 Myr ago when even the Sahara was glaciated. Of course at that time it was in the position of the South Pole'.

Almost all the authors are American and they occasionally forget that the US is not the world, but this is an inevitable consequence of the real con-

cern in America over global environmental problems and of their generous support of research in the field. By comparison we in Europe tend to take a regional view and a similar collection of European papers would be dominated by the impression that pollution and sulphur dioxide were synonymous. Elsewhere in the world with but few exceptions a narrow parochialism prevails on this topic.

The second book *Chemistry of the Atmosphere* is a lively and enthusiastic textbook of chemical aeronomy. Again the theme is set in the second paragraph of the introduction. 'To a chemist the atmosphere appears as a continuous large scale photochemical experiment. As an experimental system it is unusual in being agreeably free from wall effects . . .' The book is proudly and unashamedly specialist, so much so that the authors are prepared to recognise this limitation to their approach. They say that even the contributions of biologists and poets can be illuminating, but the reader will seek in vain for any information on that part of the air in which the birds fly or where the weather is. Indeed the only mention of the troposphere is in chapter 7 where the chemistry of air pollution is considered. This is no criticism of a book which is a splendid introduction for students of chemical aeronomy and for those interested in the scientific basis of important contemporary global problems such as the alleged depletion of the ozone layer by odd nitrogen and odd chlorine.

In this fast-moving field no book can be up to date at the time of its publication. Nevertheless the care taken by the authors to emphasise what was unknown at the time of their writing makes the additions and completions of contemporary knowledge fit comfortably with their text. The authors seem to have that personal familiarity with the practice as well as the theory of their subject which makes its explanation comprehensible and a pleasure to read.

Neither of the books enlightens the current issue of ozone depletion by chlorine-containing compounds such as the fluorochlorocarbon aerosol propellants, neither do they consider the probable future issue of ozone depletion by man-induced increases of natural nitrous oxide emissions from the soil. They should be read while their contents are still relevant.

J. E. Lovelock

New books from Blackwell

The Immune System: a Course on the Molecular and Cellular Basis of Immunity

Edited by M.J. Hobart M.A. Ph.D. Vet.M.B. and I. McConnell M.A. Ph.D. B.V.M.S. December 1975. 368 pages, 115 illustrations. Paper, £5.00

The Ecology of Resource Degradation and Renewal

Fifteenth Symposium of the British Ecological Society, edited by M.J. Chadwick and G.T. Goodman. 1975. 460 pages, 100 illustrations. £12.00

The Biochemistry of Steroid Hormones

Edited by H.L.J. Makin M.A. Ph.D. A.R.I.C. 1975. 310 pages, 194 illustrations. £16.50

River Ecology

Studies in Ecology Volume 2, edited by B.A. Whitton M.A. Ph.D. 1975. 756 pages, 149 illustrations. Paper, £18.00

Textbook of Human Genetics

G.R. Fraser M.A. M.D. Ph.D. and O. Mayo Ph.D. B.Sc. 1975. 532 pages, 80 illustrations. Paper, £9.75

Island Biology illustrated by the Land Birds of Jamaica

Studies in Ecology Volume 3, edited by D. Lack F.R.S. Sc.D. December 1975. 416 pages, 80 illustrations. About £12.50

Biogeography: an Ecological and Evolutionary Approach

C.B. Cox Ph.D. and P.D. Moore Ph.D. Second Edition, December 1975. 208 pages, 80 illustrations. Paper, about £4.00

Introduction to Microbiology

Basic Microbiology Volume 1, by J.F. Wilkinson M.A. Ph.D. Second Edition, 1975. 128 pages, 47 illustrations. Paper, £2.25

The Genetics of Algae

Botanical Monographs Volume 12, edited by R.A. Lewin. January 1976. 320 pages, 75 illustrations. About £10.00

Methods in Plant Ecology

Edited by S.B. Chapman B.Sc. Ph.D. January 1976. 528 pages, 100 illustrations. Cloth about £15.00, paper about £8.00

Methods for Ecological Bioenergetics

IBP Handbook No 24, edited by W. Grodzinski, R.Z. Klekowski and A. Duncan. 1975. 372 pages, 96 illustrations. Paper, £9.50

The Principles of Microbe and Cell Cultivation

S.J. Pirt B.Sc. Ph.D. 1975. 284 pages, 89 illustrations. £10.50

Blackwell Scientific Publications

Oxford London Edinburgh Melbourne

Penguins

The Biology of Penguins. (Biology and Environment.) Edited by Bernard Stonehouse. Pp. ix+555. (Macmillan: London and Basingstoke, March 1975.) £18.50.

This book is the first in a new Macmillan series—Biology and Environment. It is a contributive work, thoughtfully edited by Dr Bernard Stonehouse, one of the world's leading penguin biologists. Dr Stonehouse has assembled twenty papers, a mixture of reviews and the results of new research, which he has grouped into six sections and which together form a very comprehensive reference work on penguin biology. Extensive bibliographies at the end of each chapter enhance the book's academic value.

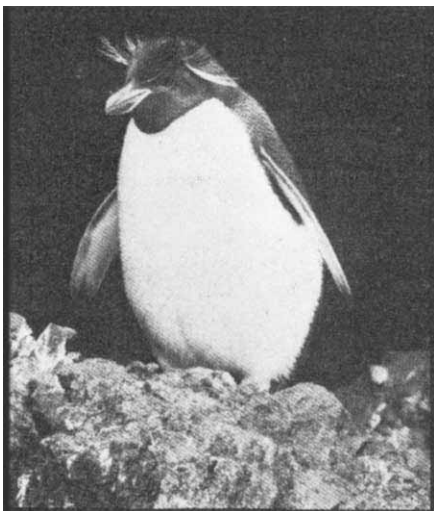
Dr Stonehouse's introduction includes a concise account of the distribution and major points of biological interest for each of the 18 penguin species. At first, this seems a little unnecessary in a book so obviously specialised, but for the non-penguin functional anatomists, physiologists, ethologists, ecologists (and others) who will also find the work of interest, this check list provides both useful background information and a ready appreciation of the size and diversity of the family. Professor G. G. Simpson begins the section on evolution and taxonomy with an updated review of fossil penguins, the first to be published since his earlier catalogue in 1946. A more recent approach to the elucidation of penguin phylogeny is reviewed in a chapter on the results of protein electrophoresis.

The section on anatomy and physiology contains five papers, ranging from a detailed study of skull anatomy, particularly in relation to feeding, to a valuable review of diving behaviour and physiology. A report on thermoregulation in the little-studied Galapagos penguin is particularly interesting. The lion's share of the book goes to the section containing three chapters on temperate and subpolar penguins amongst which the crested penguins, which have received little attention in the past, are the subject of Dr J. Warham's long contribution, the first published comprehensive account of the genus. Six chapters make up the section on breeding and population studies of Antarctic penguins and although, not surprisingly, the much-studied Adélie penguin is well represented, it is refreshing to find that information on other species has been included. Notable amongst these is an account, prepared by members of the British Antarctic Survey, of the little-known

Chinstrap penguin. A useful review of penguin predators, both at sea and on land, joins a specialised study of Emperor penguins to form a short section on predation and mortality. Adélie penguins figure again in a final section on communication and display, consisting of two chapters which provide interesting examples of different approaches to the problem of describing and interpreting behaviour.

Most of the criticisms which can be levelled at the book are explicitly felt by the editor himself. He fairly points out the absence of papers on long term population studies, parasitology and conservation, and particularly of studies at sea where, for penguins as for most seabirds, relatively little is known. Too often, contributive works of this kind are merely collections of loosely connected, highly esoteric papers but this is not the case here. Dr Stonehouse has skilfully compiled a volume which, in spite of its omissions, is sufficiently comprehensive to wear its title honestly. The book is sure to find a ready place in many libraries.

David M. Burn



Photograph: John Warham

Endangered species

Breeding Endangered Species in Captivity. Edited by R. D. Martin. Pp. xxv+420. (Academic: London and New York, May 1975.) £12.80; \$33.75. This collection of papers marks the beginning of a very much greater realisation by responsible zoos of the role that they should be playing in preventing the total loss of some fascinating and important animal species which are at risk for reasons usually related to man's rapid expansion.

The editor is to be congratulated on bringing together in a readable form, papers which vary in the quality of presentation and of content. Nearly all shed light on the task of maintaining viable breeding groups in captive conditions, especially those that are rare in the wild. A number of authors have

drawn attention to the broader issues involved in the development of breeding programmes, and some rightly demand a more pragmatic approach by those controlling animal collections to ensure the maintenance of viable breeding groups on an inter-zoo co-operation basis, thus avoiding the dangers of inbreeding and gene-pool loss. Attention is also drawn to the need to maintain rare animals in a number of collections rather than concentrating them in one area where they may so easily be destroyed by disease or other misfortune. Views are expressed as to whether the genetic changes occurring during, and possibly as a result of, captive breeding will fit animals for a return to a natural environment, especially if re-introduction is attempted after a number of generations in captivity.

Pilot projects to ascertain the problems of re-introduction are suggested, and these could as easily involve common species. In this context, it should be remembered that today's common breed could so easily be tomorrow's rarity, and that every item of information that might help to maintain these in a productive captive state should be available to those involved in this.

The Fauna Preservation Society, one of the conference sponsors, has been for many years responsible for the conservation of animals in their natural state, and has been perhaps identified with conservationists, many of whom are still unable to see the value of breeding endangered species in Zoological Gardens. The other sponsor, the Jersey Wildlife Preservation Trust, on the other hand has been at the forefront of those zoos who specialised in breeding endangered species; but in this field also there are regrettably still many zoos who do not believe their function is other than to entertain. It is the directors of these zoos that Gerald Durrell would like to see on the endangered species list.

The publication is unfortunately rather highly priced; unfortunate because it should be an essential part of every zoo director's library, and not just those Directors who are already aware of what has to be done. In a wider sense it should be on the bookshelves of all those who are deeply concerned with the future of existing wildlife and wild places.

This collection of papers may be the first of a succession of books on the important challenge of ensuring for moral and other reasons, species of animal life for the aesthetic and other pleasures of future generations. Most first efforts have their imperfections, and this is no exception, but it does form a foundation on which to expand.

R. J. Wheeler

Elephants of Uganda

Sorry, for copyright reasons some images on this page may not be available online

Elephants and their Habitats: the Ecology of elephants in North Bunyoro, Uganda. By R. M. Laws, I. S. C. Parker and R. C. B. Johnstone. Pp. xii+376. (Clarendon: Oxford; Oxford University: London, July 1975.) £15.

THIS is ecology on a grand scale. The stage is the 1,200 square miles enclosed on the west by Lake Albert and on the north and east by the great sweep of the Victoria Nile from Lake Kyoga to Pakwach. Elephant biology dominates the action, but the book encompasses the whole environment, one in which the changing scene is governed by the population dynamics of the elephant and man's relationship with these animals—in competition or conservation.

Before World War II, and for some years after it, information about elephant populations was plentiful but usually wrong. The most experienced game wardens and hunters had totally inadequate ideas about elephant numbers and movements, especially in the tall grass and forest areas such as Bunyoro. Advance has come with the development of the National Parks, and with the expansion of air travel and the use of aerial surveys. Richard Laws, a Cambridge zoologist, was foremost among the pioneers who applied modern ecological methods to populations of large mammals. He came to Africa after working on whales and seals and has since returned to his Antarctic studies, so that one may suppose him to be attracted by the big beasts and the romance that attaches to them. His approach, however, is matter-of-fact and his research is distinguished for its intellectual rigour allied to a grasp of administrative and economic problems. In this book he is assisted by a wildlife manager who was born in

East Africa and a forester who has been particularly interested in the Bunyoro district. The result will serve not only as a mine of information but also as a model of what a study of this sort can embrace.

This book is not about all "Elephants and their Habitats". The subtitle perhaps more strictly describes the content, but it is undeniably relevant to all elephant management. I think it is safe to say that there is no other mammal population in which growth, nutrition, habits, reproduction, age structure and behaviour has been so thoroughly studied in relation to climate, flora and fauna and land-use of its habitat. In north Bunyoro, "by mutual agreement between the individuals, departments and companies involved, all the relevant information has been pooled to form the basis of this book".

There are no adventure stories of life on safari here, and this is not a 'coffee table' volume. It is true practical science, an authoritative account, detailed and for practical purposes complete, for the use of students and the guidance of administrators. After reviewing the history of the region and man's use of it, the authors describe its flora and fauna in some detail before embarking on six chapters of elephant biology. There follows a chapter on the changes in habitat associated with past management and a cropping programme involving both elephant and hippo. In the final chapter the authors put forward recommendations based on their analysis of the ecological factors involved—recognising aesthetic and cultural considerations, but regarding economic considerations as "of overriding importance". Many will judge this book indispensable. **John S. Perry**

Just published

Third Symposium on the Chemistry of Nucleic Acids Components

(Special Publication No. 1 of Nucleic Acids Research)

Contains 47 papers presented at the 3rd Symposium on the Chemistry of Nucleic Acids Components held at Liblice Castle, Czechoslovakia October 8-12, 1975.

Partial Contents:

The preparation and properties of some 5-substituted uracil derivatives A.S. Jones and R.T. Walker; A facile synthesis of 5-(perfluoroalkyl)-pyrimidines D.Cech, et al; Isomerisation, alkylation, cyclisation and molecular structure of glyoxylic acid semicarbazone derivatives H.Hřebaběcký, et al; Purine α -D-ribosides and α -deoxyribosides. Syntheses and reactions H. Rokos, et al; Direct synthesis of 5-azapyrimidine ribonucleosides A. Piskala, et al; Synthetic approaches to O-glycosyl-ribonucleosides K. Kitahara, et al; Synthesis of allopurinol-ribosides E. Cuny and F.W. Lichtenthaler; A selective bromination of thymidine D. Bärwolff and P. Langen; Total syntheses of 'aspiculamycin' and gougertin F.W. Lichtenthaler, et al; Synthesis and properties of thiolumazine nucleosides I. Southon and W. Pfeleiderer; Nucleoside transformations. Conversion of guanosine 2', 3'-ortho-ester into deoxy and epoxide nucleosides R. Mengel and W. Muhs; The preparation of derivatives of 2,5-dideoxy-5-fluoro-D-ribofuranose M. von Janta-Lipinski, et al; Study of the synthesis of 5-alkyl and 5-halogen substituted 2'-deoxyuridines L. Otvos, et al; New benzyl blocking groups in nucleoside chemistry W. Hutzenlaub and W. Pfeleiderer; Protecting groups in nucleoside syntheses. I. Acetylation and cleavage of nucleosides J. Beranek and H. Hřebaběcký; Protecting groups in nucleoside syntheses. II. 2', 3'-O-Carbonyl derivatives of uridine and 6-azauridine P. Drašar, et al; Studies in C-nucleoside synthesis J.G. Buchanan, et al; A novel synthetic approach to carbocyclic ribonucleoside analogues A. Holý; Synthesis of polynucleotides in the study of structure-function relationships in the promoter-region of a tRNA gene H.-J. Fritz and H.G. Khorana. £6.00 US \$15.00 192pp October 1975



Information Retrieval Limited,
1 Falconberg Court,
London W1V 5FG, UK

Information Retrieval Inc.,
Suite 907,
1911 Jefferson Davis Highway,
Arlington, Va. 22202, U.S.A.

Mary Evans Picture Library

Prospects of the sea



Miraculous Draught of Fishes by Raphael, Courtesy V. & A. Museum

It can be very salutary to be reminded that nearly 50 years have passed since one became a student, in 1928, the year in which the first edition of "Russell and Yonge" appeared. Well before that I had been introduced to Frederick Warne's well-known (and well-read) *Wayside* and *Woodland* books, but in several respects the appearance of *The Seas* must have marked a high point in the series. This compact book provided, as nothing else had done, for both layman and student, a concise view within one cover of what was then known of the seas and the life they maintain, with a glimpse of the ways in which they were being investigated. For the beginner, and later, the book was as fascinating as it was useful and, as in the whole series, the lavish illustration was a particularly valuable feature. Although it would not be correct to suggest that for this student it was instrumental in deciding on a career (there were other influences) it had its place—and the privilege of being able to listen to the authors at the Bristol British Association meeting in 1930, following their return from

the Great Barrier Reef Expedition, also played its part. These two authors must undoubtedly have influenced many in their choice of a career, and aided far more in acquiring a sense of perspective about the life, largely hidden, that inhabits the greater part of the earth's surface.

Very rightly, the book sold well, with a second edition in 1936 (two reprints) and a third in 1963. Now a fourth and extensively revised edition has been produced in a larger format. As the blurb reasonably implies, not many co-authors are fortunate enough (in several ways) to deserve, and secure, such treatment. Not many co-authors, of course, have themselves contributed so much by way of original work (in remarkably complementary ways) to the progress made in marine research over 50 years. Both are still contributing and it is no mere coincidence that each should have been knighted for his service to science.

Their new book is still presented within the old, and very adequate, plan but it is brought up to date in many places. Indeed, collation of the latest edition with the old is a fascinating task, and it is a tribute to the care given to the earlier editions that so much can stand today, mainly with supplements rather than major alterations. There are, however, two new chapters—one on "Sensory Perception" and another on the "Influence of Man on Marine Life"—and the earlier chapters on "Shellfish" and "Products from the Sea" have been brought together under the title "Marine Exploi-

tation and Cultivation". So much has taken place, and been discovered in marine research since 1927 that, in a still compact introduction only some of the highlights can be revealed, and interesting discussions might be developed on just what is (and will be) held to be the most significant. For example, there might have been a case for saying a little about the new knowledge gained concerning continental drift, and inferences it may have for life in the seas. Again, although the Torrey Canyon and Santa Barbara episodes are mentioned, there is no reference to taking oil from under the sea in the new chapter on the "Influence of Man", although this is already influencing marine life, and will do so more and more, as also many of the men who earn their living by exploiting the seas in other ways. But where should one stop in what is necessarily an introduction to a vast subject?

This edition also has a largely new set of illustrations, for many of which the authors are indebted to Peter Stebbing, although several of the "classic" plates have been retained, together with a number of photographs by D. P. Wilson. The earlier frontispiece, however, showing a moderately equable sea has been replaced by a striking dust-cover entitled *Rough Sea!*

This edition should prove as valuable and stimulating as ever. It is well produced and readable in both senses of the word. Even at £6.95 *The Seas* is still, in these days, a good buy.

This book† is the latest addition to numerous volumes on the nature, life and problems of the sea which have appeared over the past few years. Dr Bellamy, however, has his own approach to the subject. Starting with a general description of the oceans with a very adequate account of the principal factors influencing sea and shore as environments for life, he proceeds with descriptions of the various forms of marine life and so to some account of the oceans as a source of food and other resources with the effect of man on this, largely by way of pollution.

This book is intended for the general reader who will get a very adequate general idea about the oceans from the initial chapters which include some account of the probable origin of life with an account of cell and plant life in the sea, leaving a description of animal life to occupy the following 16 chapters, almost two thirds of the book.

Here Dr Bellamy essays to describe the full range of marine invertebrates

**The Seas: An Introduction to the study of life in the Sea.* Sir Frederick S. Russell and Sir Maurice Yonge. Pp. x+283+48 plates. Fourth edition, completely revised and extended. (Warne: London and New York, September 1975.) £6.95 net.

†*The Life-Giving Sea.* By David Bellamy. Pp. 320. (Hamish Hamilton: London, September 1975.) £5.75.

to a general reader and it is clearly on his probable degree of success in this venture that any estimate of this book must largely be based. Although aided by numerous black and white and coloured illustrations, one still wonders to what extent he will accomplish his purpose.

The sponges, to which he possibly devotes too much attention, emerge as much less organised than they really are. The coelenterates get more judicious treatment although there is confusion between the stinging coral, *Millepora*, and the very different blue coral, *Heliopora* and the plate opposite page 65 showing an unnamed green coral is mystifying. The only chlorophyll in corals is contained in the brown zooxanthellae. The mushroom coral, *Fungia*, cannot "move about on the bottom"; it can only (if small enough) be carried about by water currents, although it can right itself if turned over and uncover itself when buried under sand.

The author is clearly fascinated by ctenophores (comb jellies), describing not only British species but the glorious Mediterranean Venus' Girdle and even the exotic *Coeloplana* and *Tjalfiella*. But this is unfortunately balanced by a very inadequate treatment of the far commoner and vastly more important Crustacea and Mollusca, his accounts of which one feels would only give a confused idea to any previously uninstructed reader. There is no mention of the ubiquitous amphipods and isopods; moreover crabs, lobsters and shrimps do not have nauplius larvae.

The structure of a bivalve mollusc is only indicated by an inadequate diagram and indeed throughout there is little mention of benthic organisms generally (apart from fixed algae) in spite of their fundamental importance in marine productivity. Echinoderms are better treated although the arms of brittle stars are surely not "festooned with tube feet".

There is much better treatment of the fish where, of course, even an unexperienced reader can meet the author part way. The major forms and modes of life are well described in the text and illustrated in the figures. Dr Bellamy continues through marine reptiles and birds to sea cows (a pity he says so little about these, with only an uninformative photograph of a manatee) to the whales, and a very interesting account of the work of Gerald Kooyman on the Weddell seal. As to the Californian (rather than the "southern") sea otter, the story is now changing, with the protected animal devouring every available invertebrate, including worms!

The final chapters cover marine productivity, including coral reefs, with the extent to which this is being affected

by man (although whether man's activities are responsible for the current plagues of coral-destroying crown-of-thorns starfish is dubious). But here Dr Bellamy most fittingly emphasises the significance not merely of the evolution of diverse animal forms but of "an integrated living system that mirrors the potential of the environment". One also approves his description of "pollutants" as "enrichers, suspenders, cloggers and killers" and his discussion on the relative importance of these categories.

Criticism could be made of various statements including the strange confusion—at any rate to one of this reviewer's age—of Rider Haggard instead of Conan Doyle as the originator of the *Lost World*. The plumose anemone is repeatedly termed "plum-

rose" and has *Gonyaulax* now become "*Gonyaulux*"? A final criticism concerns the author's tendency to write down to a popular level, to refer to leeches (beautifully adapted animals) as "nasty, horrible, revolting", to edible Crustacea as "mouth watering", and the use of "mind boggling" elsewhere.

Nevertheless this book has the major virtue of enthusiasm, reflecting so much of what David Bellamy has personally seen and done. He makes admirable use here and there of statistics, and the illustrations, although inevitably varying in quality (the one claiming to represent a fiddler crab surely doing so in error), are generally both apposite and informative. And finally, this is a most pleasant book to read.

C. M. Yonge

Dynamics of fishing

Marine Ecology and Fisheries. By D. H. Cushing. Pp. xiv+278. (Cambridge University: Cambridge, London, New York and Melbourne, July 1975). £9.00; \$27.50, hard cover: £3.90 paperback.

FEW attempts have been made in the past to bring together the population dynamics approach to fisheries science with the more fundamental aspects of marine ecology. Fisheries scientists concerned with heavily exploited stocks have, naturally, been preoccupied with stock assessments, fishing mortality and recruitment and have thus been concerned largely with adult and juvenile fish.

Varying recruitment to individual year classes of fish has long been recognised as a factor of major importance. The events which lead to variations in recruitment, however, are of such complexity that they have understandably discouraged efforts to integrate them into models of fish populations. They, in fact, embrace virtually the whole of marine ecology. Nevertheless, if we are to reach any real understanding leading to an ability to predict, they cannot be ignored.

Dr Cushing's book embraces material he used in a series of lectures at Oregon State University and I, personally, am sorry I did not hear the lectures. His competence in presenting the subject in written form makes it readily assimilable, and the presentation is a pleasing example of the competence of Cambridge University Press.

The first four chapters are devoted to a useful condensation and appraisal of information, widely scattered in the literature, concerning the process

of organic production in the sea. Space and time variations in the broad ocean areas are dealt with first, then the production cycles in the highly productive but unstable upwelling and divergence regions. The role of nutrients is then considered and, finally, the various attempts which have been made to model organic production in the sea are reviewed.

The next three chapters assemble information about fish stocks and migrations, their growth and mortality, and population dynamics. Again, this is a valuable condensation of a great deal of material widely scattered in literature from all over the globe.

The author then embarks on an exercise integrating our knowledge about the stocks of herrings off northern Europe with the organic production cycles—establishing a convincing correlation. Herring are planktonic feeders but behind the simplicity of this statement, the true complexity of the foodweb which culminates in herring is perplexing. It is illustrated in a critical feeding path diagram and the author explores the approaches which can be adopted to express the relationships in terms which can be handled in models.

Temporal changes and the influence of climatic factors are considered in the two following chapters and the book concludes with a discussion of the interaction between fisheries research and marine ecology, carrying a clear message that they can be mutually beneficial.

Students will find the book extremely useful and marine ecologists and fisheries biologists will broaden their outlook by consulting it.

R. I. Currie

Fruition of flowering plants

Sorry, for copyright reasons some images on this page may not be available online

The Morphology of Angiosperms: The Structure and Evolution of Flowering Plants. By K. R. Sporne. Pp. 207. (Hutchinson: London, February 1975.) £3.50 cased; £2.00 paper.

THE study and interpretation of plant morphology is a subject which captured the imagination, and occupied much of the research effort, of botanists of the nineteenth century. Yet within the last few generations the emphasis of teaching and research has swung away from the structural and toward the physiological, biochemical and ecological aspects of the subject. Although such emphases are bound to change as new techniques and ideas come to prominence, it is sad that so many of those who teach in our schools and universities have come to regard all matters structural as the descriptive studies of a former era and therefore irrelevant to their needs. Even the word 'descriptive' is held in disrepute in some quarters. Undoubtedly the lack of a good modern synthesis of morphological concepts has contributed to this situation and for this reason Sporne's trilogy of morphological texts is especially to be welcomed.

This final book, dealing with the angiosperms (previous ones have covered pteridophytes and gymnosperms), couples a careful selection of information with a lively and readable style—

both necessary attributes of a book which seeks both to enlighten and to interest the student. The arrangement of its contents is traditional, dealing with embryology, then the major structural features of the angiosperms, meristems, root, shoot, pollen, ovules, seeds, and so on. Yet the material of this book is conveyed in a far from traditional manner. There is a strong underlying theme of evolutionary trends, which both provides a unity to the examples quoted and also serves to raise those basic questions of origins which should be central in the minds of all biologists. Sporne's book excels in that he is prepared to raise these questions and attempts to answer them even at the risk of overgeneralisation.

The paucity of fossil records relevant to angiosperm phylogeny means that comparative morphology is still the most useful approach to the questions of evolution within the group. Sporne describes the modern numerical work in this field and, although he recognises its weaknesses, can unashamedly state that "a policy of caution would take half of the fun out of research". Perhaps it is the overcautious approach of his predecessors which has taken much of the fun out of plant morphology, but this lively, informative and entertaining book should succeed in injecting some renewed vigour into this neglected subject. It will be welcomed, and should be read by all students and teachers in the field of plant biology.

P. D. Moore

Science as an obstacle course

Problems of Scientific Revolution: Progress and Obstacles to Progress in the Sciences. (The Herbert Spencer Lectures 1973.) Edited by Rom Harré. Pp. vi+104. (Clarendon: Oxford; Oxford University: London, July 1975.) Paper £1.75 net; Boards £4.00 net.

I MUST be honest. I have never actually managed to get around to reading much philosophy of science, except for Medawar in passing. My reactions to this book are therefore probably akin to those students who listened to the 1973 Herbert Spencer lectures recorded therein. I hope it is not through naivety that I found most of the lectures coherent and stimulating.

The book has six contributors ranging from practising scientists to professional philosophers. The mixture works well, allowing a wide-angle approach to the subject. As I cannot believe that it is a *sine qua non* of thinking scientists that they subscribe

to the views of Sir Karl Popper, however, it is perhaps surprising that four of the six writers are self-confessed Popperians, another one is guru Popper himself, and only B. F. Skinner does not pay explicit homage.

The progress of science is viewed at its most practical level by W. F. Bodmer. He exemplifies the external factors, especially ethical and eugenic, that modulate the progress of genetics and concludes with rather hackneyed advice to scientists on how to assume a socially responsible position. Sir Hermann Bondi is more concerned with the internal forces that operate. He reveals, again by example, the kind of fits and starts involved in any scientific advance and correctly emphasises the reliance of progress on new technology.

The development of evolutionary theory is Jacques Monod's theme. He shows how the coming of molecular biology has not only added unpredictable layers of flesh to the bones of Huxley's synthetic theory but has at the same time converted it into a theory fully liable to Popperian falsification. He also attacks his critics who cannot come to terms with the advent of man by chance, and not by necessity. B. F. Skinner is even more critical of those who cannot overcome the obstacles he sees to the progress of behavioural science. Their fault, he argues with clarity, is in being trapped by an introspective, reductionist approach. Unfortunately he fails to develop his own alternative approach and its controversial application to, for example, behavioural modification.

The least tangible of the contributions is that in which J. R. Ravetz suggests that the way to salvation for the present condition of science lies in the spirit of Francis Bacon. The longest essay, replete with footnotes, is Popper's. It is divided into two parts. The first is concerned with his fallibilist theory but with several unexpected diversions. The second part deals with hindrances to scientific progress—economic factors ("big science may destroy great science"), intolerance plus dogmatism and, most pernicious, the danger of allowing scientific revolutions to become blurred by ideological entrenchment or the fashion-mongering of intellectuals.

This book is ideal for undergraduates or postgraduates. By and large it avoids being doctrinaire and is sufficiently down to earth to have a lasting and beneficial influence on its readers.

Peter Newmark

Science Between Culture and Counter-Culture. By C. I. Dessaur, Aren Naess, Everett Reimer, Hans J. Eysenck and A. G. M. van Melsen. Pp. 108. (Dekker and van de Vegt: Nijmegen, 1975.) Dfl. 19.50.

THE debate that has developed in

recent years between the proponents of culture and counter-culture has often tended to focus on science as the chief target for criticism from all sides. This issue was at the centre of the public exchange of views organised in 1974 for the tenth Lustrum of the Catholic University of Nijmegen, in the Netherlands. Five of the papers presented on this occasion have now been published: a general introduction by Professor Desaur (Nijmegen); an indictment of science on 13 different counts by Dr Arne Naess (Oslo); an autopsy of the education system—"the school is dead"—in the context of world wide transformation of culture by Dr Everett Reimer (Puerto-Rico); a defence of science against indiscriminate rejection which often chooses to ignore uncomfortable facts rather than learn to live with them, by Professor Eysenck (London); a reflection on the place of the Catholic university between culture and counter-culture by Professor Van Melsen (Nijmegen).

Critics of an enterprise such as science which exerts enormous influence on all aspects of society can always find something on to which they can latch. Defenders, on the other hand, can be quick to point out the undeniable benefits arising from scientific research; as a last resort, they can even denounce technology as a monster bred by the power structure—but for which scientists themselves will not claim responsibility, their concern being the quest for knowledge, and the quest for knowledge only.

When conducted on this level—which is more or less that of the papers presented here—the debate on the pros and cons of science is never-ending and inconclusive. It is true that each society has the science it deserves, and not surprising that "science has become the whipping-boy" (Eysenck) of those who are most critical of a society which has founded wealth and might on the exploitation of knowledge. Certainly, not all scientists have been indiscriminate and uncritical servants of that State, but most have.

The debate between culture and counter-culture seems at times merely to reformulate some of the fundamental issues discussed by C. P. Snow many years ago: why do we have two cultures—stemming from the natural sciences on the one hand and from the humanities and social sciences on the other—apparently incapable of nourishing each other? Why is it that the scientific establishment sometimes presents the appearance of a new theocracy? Why has the scientific enterprise tended to become an element of consolidation of the *status quo*, rather than an instrument of change?

These questions remain unanswered.

Georges Ferné

Source of meteorite material



Meteor trail (Great Bolide), Courtesy RAS

Carbonaceous Meteorites. (Developments in Solar System and Space Science, vol. 1.) By Bartholomew Nagy. Pp. xiv+747. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 210; \$30.95.

RETURNED lunar material apart, carbonaceous meteorites have received more intensive study per available unit mass than any other natural assemblage of minerals. The unique characteristics of carbonaceous meteorites marked them out from all other types of meteorite as soon as detailed studies began in the 19th century. The French chemist, Berzelius, examined the Alais fall—the first carbonaceous meteorite to be recognised—and reported: "These stones are different from all other meteorites because they look like solidified clay and because when they are placed in water they disintegrate and give off a clay-like odour."

Dr Nagy includes Berzelius' report, along with several other early accounts of carbonaceous meteorites (one unfortunately dated 1984), in his lengthy and detailed survey of these objects. The report continues: "The question arose in my mind, does this carbonaceous earth contain humus or a trace of other organic substances? Could this give a hint to the presence of organic formations on other planets?" We have here, at the beginning of work on carbonaceous meteorites, a statement of one of the

major reasons for the concentration of attention on them during recent years. They pose the question of whether any of the carbon compounds they contain can be considered biogenic in origin.

The debate over the answer has revolved round two distinct points: the origin of their complex organic molecules and the nature of the so-called 'organised elements'. Dr Nagy, who has been deeply involved in the investigation of both aspects, devotes over half of his book to this debate. He explains clearly the problems entailed in deciding about origin from physicochemical analysis alone. As he remarks in passing: "Were it not for the fossil remnants in coal, one would most likely still argue its origin." Above all, there is the problem of contamination. Since the compounds of interest are present only in trace amounts, minor contamination can vitiate the results obtained. "A few milligrams of dust which have fallen on and become mixed with 1 g of meteorite, would significantly alter the original hydrocarbon distribution and introduce contaminations which could be mistaken for indigenous compounds." It is hardly surprising that, although general opinion favours an abiogenic origin for the carbon compounds, definite proof is difficult to find.

The agreement that the 'organised elements' do not reflect indigenous life forms is more firmly based. But as Dr Nagy points out, the danger of judging from morphology alone is that judgement may be affected by context. He recounts: "the author once collected unicellular, blue-green algae, killed them in an organic liquid, made a microscopic preparation, and showed it to an experienced mineralogist whose speciality was meteorites. This scientist identified the dead algae as mineral grains from the Orgueil meteorite."

The second important reason for the attention paid to carbonaceous meteorites stems from the belief that they may contain some of the earliest solid material in the Solar System. The chemical, mineralogical and petrological studies that have been encouraged by this belief are described in detail by Dr Nagy, and take up about a third of the book. In this section, perhaps, the author's approach—"It is not the purpose of this book to evaluate the validity of reported findings and theories; the reader will have to do this to his own satisfaction"—works less well. The interpretation of the data is so uncertain that rather more guidance would have been valuable. In all, however, this book provides an eminently useful account of the present state of knowledge of carbonaceous meteorites, and can be recommended as a reference source.

A. J. Meadows

Jurassic environments

Jurassic Environment. (Cambridge Earth Sciences Series.) By A. Hallam. Pp. ix+269. (Cambridge University: Cambridge and London, August 1975.) £11.00.

THE Jurassic System has a special place in geological history as it provided the touchstone for early concepts in stratigraphy and biostratigraphy. Just before he died in 1956, W. J. Arkell, in his *Jurassic Geology of the World*, reviewed the global status of these studies in the final of several of his works which are already, in the best sense, classics of geological literature. The subsequent two decades have been revolutionary ones for the geological sciences, with quite new approaches possible through palaeomagnetism and the concept of seafloor spreading: for the Jurassic, too, substantial work has been done in formerly little known areas. The time may be ripe for a dynamic synthesis on the geography, biogeography and ecology of the period, collating and the interpreting of the changing Jurassic environments.

Over the past two decades Dr Hallam has enlivened Jurassic specialists with a range of novel contributions on various aspects of Jurassic environments, contributions which often emphasised the inadequacies of currently held hypotheses rather more than they provided acceptable explanations of their underlying problems. The stimulating role of these in Jurassic studies, especially to a younger generation, has been considerable. It is therefore particularly welcome to have a summary which brings together his views on the sediments, tectonism, sealevel movements, climate, and biogeography of the period.

The most satisfactory parts of the book are where he attempts a rigorous analysis of Jurassic tectonism, sealevel changes and ammonite provincialism, even if length does not permit an exhaustive treatment. These are marked advances over anything which could have been written twenty years ago. Less satisfactory are the instances, especially in the several chapters on various sediment types, which raise problems common to most geological periods. Here, quixotic tilting at various hypotheses when space is not available to deal thoroughly with any of them is not helpful.

Nonetheless this is a refreshing and valuable contribution for those with any interest at all in the Mesozoic Era. For Jurassic specialists the attempt at updating Arkell's work, and the long bibliography of more recent work, will

be welcome. Students (if any can afford to buy it) will appreciate the breadth of view presented in a work which will add some sparkle to their labours.

How vividly a comparison of this work with Arkell's 1956 volume brings out contrasts in the scientific approach: Hallam delighting in controversy and speculation, Arkell cautious and scholarly. The one giving a stimulating attack on problems, the other working most to ensure a sound foundation for advance. The writing of the one often repetitive and with printer's errors, that of the other always of systematic and elegant prose. Science uses both, but what would Joscelyn Arkell have thought on the fulsome dedication of a volume to him which spells his name incorrectly even on the dedication page?

M. R. House

Cosmic rays

The Origin of Cosmic Rays. (Nato Advanced Study Institutes Series, Series C.) Edited by J. L. Osborne and A. W. Wolfendale. Pp. x+466. (Reidel: Dordrecht and Boston, Massachusetts, April 1975.) Dfl. 105; £39.50.

Composition of Cosmic Radiation. (Topics in Astrophysics and Space Physics, Vol. 11. By Krishna M. V. Apparao. Pp. x+86. (Gordon and Breach: London, New York and Paris, March 1975.) £5.40.

SPECULATION about the origin of cosmic rays has gone on ever since the discovery of the radiation itself, but in the early stages that speculation was inevitably somewhat wild and unproductive. A quite demoralising lack of hard facts about physical conditions in possible source regions and ignorance about the processes which could occur there gave to the discussions a metaphysical flavour, a tinge of the glamour of the occult, that they have never wholly lost.

But times have changed. Fermi's suggestion, that the particles are accelerated by collisions with moving gas clouds during their zig-zag motion through the magnetic fields of interstellar space, brought the cosmic ray question firmly into the forefront of contemporary astrophysics, and there it has remained. Yet the debate on origins is not over. The Fermi model has given way to others in which supernova explosions, supernova remnants (pulsars) and explosive events in the nuclei of our own and other galaxies have been

suggested as cosmic ray sources. The experimental data on which these rival models will be judged is accumulating steadily. There have been spectacular improvements in our knowledge of the chemical composition of cosmic rays, their energy spectra, and their directional properties. Optical and radio astronomy—and, more recently, X-ray and γ -ray astronomy—have provided much relevant information about the physical conditions in regions where acceleration and propagation of cosmic rays must take place.

The meeting organised in Durham last year by Professor Wolfendale and his colleagues, with the financial support of NATO, was planned to consider the origin of cosmic rays in this broad astrophysical context, and it is the proceedings of that meeting which are now available in book form. That publication has taken place in so short a time (less than twelve months) is something of an achievement in itself, and although at first sight the price (verging on £20) must seem high, the book is in truth a veritable bargain. The contributors are authoritative, they have written with the needs of students in mind and not solely for their fellow experts, and the material is as near up-to-date as one could hope for. The known facts about the particle radiations are summarised by Watson (Leeds), Thambyahpillai (London), Rasmussen (Lyngby), and Meyer (Chicago), whereas the related γ radiation is considered by Pinkau (Munich) and Stecker (NASA). Relevant features of galactic structure are described by Osborne (Durham) and Thielheim (Kiel), and the link between the chemical composition of cosmic rays and nuclear processes within stars is discussed by Reeves (Saclay). The arguments in favour of the three major models for cosmic ray acceleration are put forward by their best-known advocates: Burbidge (San Diego), Pacini (Frascati) and Colgate (New Mexico). With such a galaxy of authors, the book will surely become a *vade mecum* for every postgraduate student concerned with astrophysics, and a valued source of reference for others.

The second book under review, on the chemical composition of the cosmic radiation, is a much slimmer volume. The author is, once again, an authority in his field and writes to interest students as well as specialists. In principle, he succeeds. Unfortunately, he has been ill-served by the leisurely pace of book production. Important developments, duly noted in the Durham proceedings, are missing in Dr Apparao's book, which carries as its most up-to-date reference "1971—in the press". Although the book is still of interest and has its uses, the potential purchaser must reflect that the price per page is substantially greater than for the Durham proceedings, and that the information it gives him is three years behind the times.

H. R. Allan

States of matter

States of Matter. (Prentice-Hall Physics Series.) By David L. Goodstein. Pp. xii+500. (Prentice-Hall: Englewood Cliffs, N.J., March 1975.) £12.50.

THIS book is based on lectures given at Caltech as an advanced year-long course in applied physics. It is an outstanding example of the nutritious pabulum fed to American postgraduate scientists, and indeed constitutes a very meaty dish. The first chapter (about 90 pages), based largely as the author indicates on Landau and Lifshitz, provides a rigorous introduction to thermodynamics and statistical mechanics. The following chapters then deal with the perfect gas (40 pages), solids (70 pages), liquids and interacting gases (90 pages), some special states including superfluidity, superconductivity and magnetism (90 pages) and finally critical phenomena and phase transitions including scaling laws (50 pages). Each chapter concludes with some shrewd comments on bibliographical sources and some well-tryed, rather demanding examples.

Throughout the book the main themes are tackled in terms of thermodynamics and statistical mechanics. This unified, integrated treatment has many attractive features. It has the disadvantage, however, that in some cases the approach is somewhat tortuous. Again the mathematical analysis is often heavy going and rather formal. On the other hand the accompanying discussion, which is expressed in a verbal rather than a written style is always lively and communicative and reflects a first-hand mastery of the issues under review. Those who find the analysis too tough will always find the author's comments illuminating: they breathe life into the dry bones of the formal analysis and provide a fresh and often entertaining insight into the physics of the problem under discussion. There are also some agreeable personal touches—for example, on quantised entities ending in -ons, such as phonons, photons, and so on. "Even human populations have quantised units called persons." On classical theories: so called not necessarily to distinguish them from quantum mechanical, "but simply because classical is what we usually call the theory that came just before the one we believe now." On the irreversible tendency for entropy to increase he quotes the famous quatrain of Omar Kayyam ("... the moving finger ...") with the comment that "there have been many other statements of the Second Law, all of them less elegant."

The chapter on liquids, fascinating as it is, probably provides material least familiar to the student, and the physics seems to be overlaid too heavily with computational tricks for calculating the interaction of atomic clusters. Again the inadequacy of pairwise additivity could have been made more concrete if the author had compared, for example, the lattice energy of solid argon with that deduced from pairwise calculations: and if his fifth chapter had dealt with dielectrics (as well as magnetism) he could have linked the problem of additivity with the dielectric properties of matter in the condensed state. This in turn provides a bridge with the van der Waals'

properties of liquids and solids. Finally, the author quotes the opinion of some scientists that plasmas constitute the fourth state of matter: it would do no harm if applied physicists were to be taught instead about the colloidal state. This is not only of great importance, it also offers scope for some very nice physics. But these are personal opinions rather than criticisms.

Dr Goodstein's heroes, to judge from his references and comments, are Landau and Lifshitz, Mott and Jones, G. I. Taylor and Galileo Galilei. This fine sense of scientific discrimination and catholicity of interests characterise the stimulating and challenging book he has written.

David Tabor

Aged Ecuadoreans



Michaela Mendieta Quesada, aged 106

The Centenarians of the Andes. By David Davies. Pp. 128+17 photographs. (Barrie and Jenkins: London, July 1975.) £3.95.

POPULATIONS containing numerous centenarians and supercentenarians have been reputed to exist in the Ecuadorean Andes since 1825 (G. Coggeshall, 1825, *Voyages to various parts of the world*), and have recently been noted by Leaf (*Youth in old age*, 1975). This book is an anecdotal but informative study of the distribution, way of life and documentary authenticity of centenarians in Vilcabamba, who have been fairly extensively studied, at least from the standpoint of documentation by Dr Miguel Salvador for the Ecuadorean Government. Ages of 140 and 150 years have been reported, but equally remarkable is the state of physical preservation of people throughout old age. Dr Davies' book

contains no demographic tables, so that the population breakdown cannot be ascertained from it. As in Abkhasia, interest naturally focusses on the authenticity of the high age records—these seem better documented than in Abkhasian Muslims, by reason of the care devoted to Catholic birth and burial registers.

At present the high age population seems to be confined to a small enclave of mountain villages, but there are rumours of similar longevity in other parts of the Andes not yet investigated. In the villages around Vilcabamba longevity seems to affect at least three distinct population types. Possible causes of survival (genetic endowment, hybrid vigour, low calorie or low fat diet, particular dietary constituents, environment, and absence of psychosocial pressure to age) are discussed, but there is as yet no evidence to support any one of these factors, except perhaps isolation from viruses and continued acceptance of 'old' activity, which clearly operates in the field of work and of sexuality. The environment but not the diet resembles that of Abkhasia, where longevity is reportedly geographic rather than specifically ethnic, affecting Russians, Abkhases and Jews who live in the area.

Dr Davies' treatment is of necessity that of a travelogue, illustrated with some good photographs. A proper monograph giving the demographic picture and the true prevalence of centenarianism is overdue, and it is to be hoped, in view of the great interest of the model, that Dr Salvador and the Ecuadorean Government will provide us with one to amplify a previously circulated report (M. Salvador, *et al.*, 1972, *Vilcabamba, tierra de longevos*, Quito: Casa de la Cultura Ecuatorian).

Alex Comfort

Aspects of oceanography

Introduction to Marine Geology and Geomorphology. By Cuchlaine A. M. King. Pp. 309. (Edward Arnold: London, 1975.) £9.90.

Introduction to Physical and Biological Oceanography. By Cuchlaine A. M. King. Pp. 372 (Edward Arnold: London, June 1975.) Boards £11; Paper £5.50.

OCEANOGRAPHY has progressed in the last decade to an even greater extent than most sciences. This is reflected in the fact that Professor King when preparing a second edition of his well-known textbook *Oceanography for Geographers* found it necessary to divide it into two volumes. The first of these deals with marine geology and geomorphology. The book commences with an introductory chapter, which skimpily covers topics as various as deep-sea sediment sampling, diving, station fixing and ocean resources. It continues with a chapter on the structure of the ocean basins in which rather restricted coverage is given to ocean spreading and plate tectonics. The next two chapters, which deal with the continental margins and the morphology of the open sea are very good and probably the most useful in the book. The fifth chapter deals with deep-sea and near-shore sediments and their rates and mechanisms of formation; there is, regrettably, little on their chemistry.

The book closes with a chapter on the origin of ocean water and changes in sea level; the treatment of the former is extremely perfunctory, and no mention is made of the origin of the dissolved solids which give seawater its special character. In general, although some effort has been made to include recent work, this has been done in a rather unselective way. It is particularly disappointing to find no discussion of the results obtained during the Deep-Sea Drilling Program as this has done so much to revolutionise our ideas on marine geology and sedimentology. Although this book can be recommended for students of geography requiring an introduction to marine geomorphology, it is likely to be of much less value to those seeking background reading in marine geology, because of its superficial treatment of the subject.

The second book is an uneasy union between physical and biological oceanography. It commences with an introductory chapter which does not relate well with the following chapters and the purpose of which is obscure, particularly when related to chapter 8. Then follow chapters on physical oceanography

dealing with the waters of the ocean and their circulation, tides and waves. Much of it is dealt with in a purely descriptive fashion. On the occasions, particularly in chapter 3, when explanations of physical phenomena are offered they are often garbled to such an extent as to suggest a basic lack of understanding on the part of the author (for example, the first complete paragraph on page 141 and the first three paragraphs on page 68 on westward intensification). There are also a number of unjustifiably bold statements of "fact". Thus, the tsunami wavelength (not a well-defined quantity) is stated to be 900 km on page 8 and 160 km on page 9, but surely not in Loch Lomond (page 190). The discussion of the heat budget on page 30, based on work done in 1936, makes no mention of the fact that the individual contributions to it are still not known with any accuracy. On page 33 the impression is given that the only minor elements present in sea water are silver, gold and radium.

The book is out of date in a number of important respects; thus, in chapter 5 the discussion on the observed wind-wave spectrum makes no mention of JONSWAP and the Pierson-Moskowitz spectrum; nor are the currently accepted ideas on longshore and rip currents presented. In many instances important terms are left undefined and even undescribed (for example, PCB, Coriolis force, primary production, and salinity, the latter being only related to chlorinity (itself undefined) by an out of date formula). The text is strewn with minor undefined terms, of which there are six examples in the first paragraph on page 141.

The next two chapters consisting of 94 pages in all, are concerned with biological matters. The first of them deals in a very disjointed way with marine production at all trophic levels. In spite of its key importance primary production is dismissed in about 6 pages. The following chapter deals with the biological exploitation of the ocean, and is primarily a scrappy account of fishing and whaling. The final chapter on "Uses and problems of the oceans", includes subjects as diverse as oceanic pollution, conservation and law of the sea. The author's literary style is an awkward one, and occasionally sentences have to be read several times before they can be understood. Although this book may perhaps be of value for geographers, in the reviewer's opinion it has little to offer to prospective oceanographers or marine biologists, because of its superficiality.

J. P. Riley

Materials and Technology

The publication of Volume 8 in September 1975 sees the completion of this systematic encyclopedia which has been produced for people who want to know about materials, including those who have no advanced training in science or technology. Within each volume each chapter has been written by a specialist or a group of specialists so as to represent a comprehensive account of the subject, which will be of use not only to the general reader but also to those who work with materials in their professional capacity. It will also fulfill a useful function in education as a link between formal textbooks and the practical application of academic learning.

Volumes are:

Air, water, inorganic chemicals and nucleonics
Non-metallic ores, silicate industries and solid mineral fuels
Metals and ores
Petroleum and organic chemicals
Natural organic materials and related synthetic products
Wood, paper, textiles, plastics and photographic materials
Vegetable food products and luxuries
Edible oils and fats, animal food products, and material resources: general index.

Each volume is priced at £20.00 but there is a reduction of £2.00 per volume on all orders for the complete set.

We have produced a 16 page brochure on *Materials and Technology* which includes full details of the contents of each volume. If you would like to receive this please write to:
 Room 504, Longman House,
 Burnt Mill, Harlow,
 Essex CM20 2JE.



Longman

review article

Mammalian plasma membranes

Mark S. Bretscher* & Martin C. Raff†

1975 is the fiftieth anniversary of the proposal, by Gorter and Grendel, that biological membranes are based on a lipid bilayer. Now well established, this proposal, like the DNA double helix, was a major breakthrough in molecular cell biology. Here we discuss current concepts about the molecular composition, organisation and behaviour of the plasma membranes of mammalian erythrocytes and nucleated cells.

CELL membrane structure and function are intimately linked to each other and to much of biology. The plasma membrane is the most experimentally accessible and, in many respects, the most important of the cell's membranes. It maintains the internal milieu of the cell and has a vital role in intercellular communication. In this overview, we discuss the general features of normal mammalian plasma membrane architecture and behaviour that have been gleaned from recent experiments on lipid bilayers; erythrocyte ghosts and nucleated cells.

The lipid bilayer

The three classes of lipids in cell membranes, phospholipids (which comprise the majority), neutral lipids (mainly cholesterol) and glycolipids (largely glycosphingolipids), usually constitute almost half the plasma membrane mass. They are all amphipathic, having a hydrophilic (polar) and hydrophobic (non-polar) end. Phospholipid molecules have a polar head group and two hydrophobic hydrocarbon tails of variable lengths and degrees of unsaturation; when placed in an aqueous environment they spontaneously form bilayers, burying their tails and leaving the head groups exposed to water. Low angle X-ray diffraction studies of various membranes show that the bulk of the phospholipids in these membranes are arranged in the form of a bilayer¹; this provides the structural framework of the membrane¹⁻³.

An important advance in understanding membrane structure has come from studies of the physical properties of lipid molecules in synthetic bilayers and selected biological membranes. A variety of techniques, including nuclear magnetic and electron spin resonance, have been used to measure the motion of individual lipid molecules or their different parts^{2,4}. These show that lipid molecules in artificial bilayers readily exchange places with their neighbours in their own monolayer⁵, but very rarely migrate from one monolayer to the other⁶, a process called "flip-flop". Thus, lateral diffusion of a lipid is rapid, each molecule exchanging with its neighbour about 10⁶ times a second (ref. 7); in contrast, neighbour exchange across the bilayer by flip-flop occurs less than once a fortnight^{8,9}. Nor are individual lipid molecules rigid; their hydrocarbon chains flex and the degree of flexion may be greatest near the

centre of the bilayer and least adjacent to the polar head group¹⁰. Thus the lipid component of a membrane is a two-dimensional liquid in which molecules are restricted to their own monolayer.

The fluidity of a bilayer at a given temperature is determined by its composition. An artificial bilayer of a single type of phospholipid (such as dipalmitoyl phosphatidyl choline) has a sharp and characteristic melting point (or phase transition) at which it changes from its liquid state to a rigid crystalline bilayer (or gel phase)⁴. This temperature depends on the nature of the phospholipid head group and on the length and degree of saturation of the hydrocarbon side chains; as might be expected, a high melting temperature is found for lipids with long hydrocarbon chains, whereas the introduction of *cis*-double bonds into these chains (which makes them difficult to pack next to each other) leads to a low melting temperature. In artificial bilayers containing a mixture of phospholipids there is a progressive phase change over a broad temperature range, indicating that a "phase separation" into clusters of gel and liquid occurs^{4,11}.

When cholesterol is introduced into a bilayer, the plate-like steroid rings interact with, and partly immobilise, those regions of the hydrocarbon chains closest to the polar head group (leaving the rest of the paraffin chains flexible); this prevents their crystallisation⁴. Thus, in high concentration, cholesterol abolishes temperature-induced phase transitions; it has the dual effect of preventing formation of crystalline gel areas while inhibiting the overall flexing motion of the hydrocarbon chains. In this way, it imparts an "intermediate fluid condition" to the interior of the bilayer⁴.

In thinking about plasma membrane lipids, it is often helpful to draw an analogy between them (as two-dimensional solvents) and water (as a three-dimensional solvent). For example, we may wonder why mammalian plasma membranes contain a variety of phospholipids having different head groups, such as phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. In part, this may be because some membrane enzymes need specific phospholipids for normal function^{12,13}. It is well known that water-soluble enzymes often require a particular ion for activity and, in a similar way, a membrane enzyme may require an adjacent ethanolamine (rather than serine) head group as activator. Since the bilayer is a liquid, an individual phospholipid molecule could activate several different membrane enzymes over a period of time, just as an ion could for soluble enzymes. It has been reported that proteins

* Cell Biology Division, Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

† Medical Research Council Neuroimmunology Project, Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK.

which penetrate into the lipid bilayer tend to immobilise a collar of surrounding lipid molecules ("boundary lipid")^{11,12,14,15}. This is comparable with the tendency of water molecules to exist in an ordered state around the surface of water-soluble proteins.

In mammalian plasma membranes, phospholipid heterogeneity and the presence of cholesterol and protein thus modulate the fluidity of the bilayer. The biological importance of membrane fluidity is suggested by the fact that yeast, bacteria and various poikilothermic organisms change their membrane lipid composition to maintain a relatively constant fluidity in the face of changes in temperature^{16,17}.

There is increasing evidence for significant asymmetry between the inner and outer leaflets of the lipid bilayer of human erythrocytes. Glycolipids seem to be confined to the outer half of the bilayer¹⁸, and radiolabelling^{19,20} and phospholipase-mediated digestion studies²¹ indicate that most of the choline-containing phospholipids (phosphatidyl choline and sphingomyelin) are found in the outer half of the bilayer, while the amino phospholipids (phosphatidyl serine and phosphatidyl ethanolamine) are largely confined to the inner half. Since the former lipids are more saturated than the latter, head group asymmetry implies asymmetry in the hydrocarbon tails as well; and because only phosphatidyl serine carries a net negative charge, whereas the other phospholipids are zwitterionic at physiological pH, there is also a charge asymmetry between the monolayers. Although it is not known whether lipid asymmetry exists in other cell membranes, there is increasing evidence that it does. Since phospholipids do not spontaneously flip-flop at an appreciable rate, it seems likely that the asymmetry arises during biosynthesis²⁰. Its biological significance is unknown.

The relationship between the lipids in different membranes of a cell is obscure. Although all membranes can deacylate and reacylate their own phospholipids, only the endoplasmic reticulum has the enzymes required for *de novo* phospholipid synthesis²², which suggests that phospholipids are synthesised and inserted into pre-existing membrane there. Furthermore, specific proteins in the cytosol exchange phospholipids between the different membrane compartments²², but how the observed differences in lipid composition between organelles within a cell are generated or maintained is unknown. In erythrocytes, where *de novo* lipid synthesis does not occur, there is a slow renewal and remodelling of cellular phospholipids through exchange with plasma lipids²³. It is not clear to what extent such exchange occurs in other cells, but most cells seem able to incorporate exogenous glycolipids into their surface membranes²⁴.

There remains a number of unresolved questions about membrane lipids which could have important implications for membrane function. For example, it is often assumed that each lipid monolayer of a membrane is under the same lateral pressure, but this need not be so. It seems quite possible that the external monolayer is under compression and the inner monolayer under tension. Such an arrangement can be visualised if we think of an area of bilayer with more phospholipids on one side than on the other, but in which each monolayer covers the same area. This is possible because lipids are rather elastic to lateral compression. Again, it is unclear to what extent there is compositional or conformational (liquid against gel) heterogeneity within each monolayer, and whether such heterogeneity would have biological significance. A change in lipid conformation can lead to a change in protein distribution in reconstituted membranes¹¹, but it seems doubtful that membrane protein distribution or function is ever normally controlled by changes in lipid conformation.

Finally, the widespread occurrence of many different glycolipids should be mentioned. In general, their biological function is obscure, although some of the gangliosides have been implicated as receptors for some bacterial exotoxins, such as cholera^{24,25} and tetanus²⁵.

Association of membrane proteins with the bilayer

More than half the mass of most plasma membranes is protein. Unlike the extended monolayer of protein on both surfaces of a membrane originally envisaged³, membrane proteins seem to be built into the lipid bilayer in a highly asymmetrical way. The ease with which these proteins can be removed from the membrane varies over a more or less continuous spectrum—from those which can be eluted by rather gentle procedures (such as lowering the ionic strength) to those which can only be removed by total disruption of the bilayer matrix. The two extreme cases are sometimes referred to as "peripheral" or "extrinsic" and "integral" or "intrinsic" proteins²⁶. In a loose sense this classification may be helpful, provided that it is not mistaken for a molecular description of how these proteins are arranged in their membranes, which, in almost all cases, is unknown. Some proteins extend across the bilayer; these are believed to have hydrophobic surfaces which associate with lipid molecules by hydrophobic interactions in the bilayer interior. To solubilise them, detergents, organic solvents or chaotropic agents are needed which break hydrophobic bonds and destroy the bilayer; even then, some strongly associated lipid may remain. Detergents, like membrane lipids, are amphipathic; they can be anionic (as with sodium dodecyl sulphate (SDS), deoxycholate (DOC), or Sarcosyl), cationic (such as Cetavlon) or non-ionic (as with the Triton, Brij and Tween detergents, Lubrol PX, Nonidet P40). Their hydrophobic ends bind to the hydrophobic regions of the proteins, largely replacing the phospholipid, and thus bring the proteins into solution as detergent-protein complexes²⁷. In some cases, the detergent (such as SDS) also binds to hydrophilic regions of the protein, causing them to unfold. Even with non-denaturing detergents, if the detergent (and remaining lipid) is removed, membrane proteins are often highly insoluble and aggregate in neutral aqueous buffers.

Membrane biochemistry has been revolutionised by the use of electrophoresis in SDS polyacrylamide gels, to separate and study solubilised membrane proteins. In this procedure, the amount of anionic SDS bound is approximately proportional to the mass of the polypeptide. The charge of the detergent overwhelms the intrinsic charge of the protein, which thus migrates according to its molecular weight when sieved by the polyacrylamide; it can later be visualised by various protein stains. Although glycoproteins can also be resolved by this technique, estimating their molecular weights from their electrophoretic mobility is misleading if they contain large amounts of carbohydrate, because the amount of SDS bound per unit mass of glycoprotein, and the hydrodynamic behaviour of the detergent-protein complex, are altered^{20,28}.

Membrane proteins of erythrocytes

More is known about the plasma membrane of human erythrocytes than about that of any other mammalian cell, mainly because it is the only membrane in these cells and can be readily isolated intact, free from contamination by other cells or organelles. Erythrocyte membranes, or "ghosts", are prepared by hypotonic lysis of red cells, which releases the haemoglobin. By studying intact cells, permeable (unsealed) ghosts, or inside-out resealed ghosts²⁹, one can study both the external and cytoplasmic faces of the membrane.

When human erythrocyte membrane proteins are studied by SDS polyacrylamide gel electrophoresis, approximately 15 major protein bands are seen, varying in molecular weight from 15,000 to 250,000. The properties of these proteins have been frequently reviewed^{20,29-31}, so they will not be discussed in detail here. Several important general points have, however, emerged from studies using labelling

reagents or proteolytic enzymes to detect which proteins are accessible on one or other (or both) membrane surface(s). (1) All of the proteins and glycoproteins are asymmetrically oriented across the bilayer, being exposed on one or both sides of the membrane. (2) Most of the protein molecules are associated with the cytoplasmic side of the membrane, and of these, most can be removed from the membrane in very low ionic strength solutions. The most abundant protein located on the cytoplasmic side is "Tekton A" (ref. 32) or "spectrin", a fibrous protein which comprises about 25% of the membrane protein mass and which is composed of two separable polypeptides (about 220,000 and 240,000 daltons each). (3) There are two major proteins exposed on the external surface; both are glycoproteins which extend to the cytoplasmic surface and thus span the membrane. No protein is found associated only with the external surface of the cell. The larger of the two proteins which span the bilayer (electrophoretic band 3) (ref. 29) is a globular protein of about 100,000 daltons; it has relatively little carbohydrate (but may be a receptor for concanavalin A) (ref. 33), and may be present in the membrane as a dimer. This protein is believed to be the red cell anion and glucose channel^{29,34,35}. The other (MN glycoprotein=major sialoglycoprotein=glycophorin) is the most completely characterised mammalian plasma membrane protein; it is 131 amino acids long and has a molecular weight of approximately 30,000 (refs 31 and 36). Its amino-terminal hydrophilic end at the cell surface carries all of the carbohydrate (about 100 sugar residues), accounting for the great majority of the cell surface carbohydrate, including more than 90% of the sialic acid, and thus most of the negative charge of the cell surface. Its hydrophilic C-terminal tail is exposed on the cytoplasmic side, while an α -helical hydrophobic segment of 23 amino acid residues spans the non-polar bilayer. This glycoprotein carries some blood group alloantigens (MN, possibly ABO) and acts as a receptor for influenza virus and some plant lectins (PHA, wheat germ agglutinin)³¹. Besides these major proteins, a number of membrane-bound enzymes are present in such small amounts that they do not show up as distinct bands on gels; nonetheless, it seems from functional studies that some of these also are situated asymmetrically across the bilayer²⁹.

At present, freeze fracture electron microscopy is the only way of visualising the interior of a cell membrane. In this process cells are fractured while frozen in a small block of ice. The fracture plane passes through the middle of the bilayer³⁷, exposing two complementary fracture faces, which are shadowed with platinum and carbon. The resulting replicas, examined in an electron microscope, reveal that the inner (A) and outer (B) halves of the erythrocyte bilayer are studded with small (80 Å) intramembrane particles. These are homogeneous in size and randomly distributed, being more dense on the inner (A) face. Since lipid bilayers and vesicles are devoid of particles unless proteins are included, the particles probably consist mainly of protein. It is likely that to be visualised as a particle, a polypeptide, either by itself, or as part of a subunit aggregate, must span the membrane and be of sufficient size to "cast a shadow". Although it is unproven which proteins constitute the particles in human erythrocytes, much of the evidence is consistent with contributions both from band 3 and from the MN glycoprotein. Thus, when erythrocyte ghost particles are induced to aggregate into clusters (see below), cell surface ABO antigens³⁸, receptors for the lectins, PHA³⁹ and concanavalin A⁴⁰, and for influenza virus³⁹ are aggregated in the same pattern as the particles. This suggests that the particles extend to the outer surface of the ghost membrane, and that most are constituted by the two major surface-exposed proteins that are thought to carry these antigens and receptors.

The principal surface-exposed proteins of erythrocytes seem to be relatively immobile⁴¹, and in this respect differ

from those of nucleated mammalian cells, in which many of the plasma membrane proteins are able to move about in the plane of the membrane. There is increasing evidence that spectrin forms a filamentous network on the cytoplasmic surface of the erythrocyte membrane and it is this that directly or indirectly holds the particles, and thus the major penetrating proteins that probably constitute them, in place⁴². Thus, intramembrane particles can be induced to aggregate in ghosts in a variety of conditions, but only in ghosts which have been depleted of spectrin; particle aggregation does not readily occur in intact erythrocytes, or in ghosts having a full complement of spectrin⁴². It is not known whether the various minor erythrocyte membrane proteins, such as Na⁺-K⁺ ATPase or acetylcholinesterase, are capable of translational mobility, or whether they, too, are immobile.

Membrane proteins of nucleated cells

The total membrane mass in nucleated cells includes nuclear, endoplasmic reticulum, Golgi, mitochondrial and various vesicle membranes. The plasma membrane can therefore be studied only after its isolation from these other membranes, or by using membrane-impermeable reagents to label surface proteins. Moreover, reliable methods for making the equivalent of permeable or inside-out ghosts to study the cytoplasmic side of these membranes are still lacking, although isolated phagosomes may well prove useful in this respect⁴³. Thus, while it seems likely that a larger variety of membrane proteins and glycoproteins will be found in most nucleated cells than in erythrocytes, little is known about their topographical arrangement in the membrane. One very large glycoprotein of cultured fibroblasts has, however, attracted considerable attention⁴⁴. It seems to have a molecular weight of about 250,000 and is accessible to the cell exterior, since it is extremely labile when intact cells are treated with low levels of trypsin. This protein is also sensitive to proteolytic attack from the cytoplasmic side of the membrane (in phagosomes, which contain an inverted region of plasma membrane) and thus probably spans the fibroblast plasma membrane⁴³. The great interest in this molecule resides in the observation⁴⁴ that it is absent from the plasma membrane of a variety of virally-transformed fibroblasts, which suggests that it may have a role in the control of cell growth.

Perhaps the most important recent discovery about the plasma membrane of nucleated cells is that many of its proteins are mobile, being able to diffuse laterally in the plane of the membrane, and, by analogy with rhodopsin molecules in retinal rod disk membranes⁴⁵, to rotate about an axis perpendicular to the membrane plane. The first suggestion of this came from X-ray diffraction studies on rod disk membranes, which indicated that rhodopsin molecules, the major proteins in these membranes, were mobile and in a "liquid" state⁴⁶. The dramatic demonstration, in 1970, that different antigens on the surfaces of nucleated mouse cells and human cells rapidly intermix when the cells are fused by inactivated Sendai virus was the first indication that proteins in the plasma membrane had considerable mobility⁴⁷. The following year, it was demonstrated that divalent antibodies, binding to immunoglobulin receptors, or to other antigens on the surface of lymphocytes, lead to the selective redistribution of the antibody-antigen complexes in the plane of the membrane⁴⁸. Three distinct types of redistribution occur. (1) Patch formation, which is the passive clustering of cross-linked macromolecules into aggregates, a process analogous to a precipitation or agglutination reaction occurring in the two dimensions of the fluid membrane⁴⁹. (2) Cap formation, the polar segregation of the cross-linked molecules from other membrane components by an active process, probably dependent on microfilaments^{48,50}. (3) Pinocytosis of the complexes, which in some cases may lead to extensive loss of

the membrane protein^{48,50}, a phenomenon referred to as "antigenic modulation"⁵¹. In some systems, shedding of the surface antigen-antibody complexes may contribute to modulation. Moreover, monovalent Fab fragments of antibodies can, in some situations, induce pinocytosis⁴⁹ and/or modulation⁵¹. Hormone-induced disappearance of cell surface hormone receptors, referred to as "down regulation"⁵², presumably represents another example of ligand-induced modulation. In each case, the process is reversible: the cells resynthesising the modulated protein when incubated in the absence of ligand. It is likely that modulation represents a normal homeostatic mechanism whereby the concentration of signalling ligand regulates the concentration of its receptors on a target surface.

Patching and pinocytosis can be induced in all nucleated mammalian cells so far studied, if an appropriate cross-linking agent is used, while capping has been demonstrated in many (but not all) cell types, including lymphocytes, granulocytes, and fibroblasts. Besides providing direct evidence for membrane protein mobility, ligand-induced redistribution is a powerful tool for mapping cell membranes. For example, it is possible to determine the relationship between two molecules, A and B, on the surface of a cell by redistributing A with anti-A antibody and seeing what happens to B; if B redistributes with A, the two molecules are associated in the membrane (or become associated after ligand binding), whereas, if B is left behind, they are independent. The two antibodies are followed by conjugating them with different markers, such as fluorescein and rhodamine. By such "cocapping" experiments, it has been shown that the major histocompatibility loci in man (referred to as HLA-A, HLA-B and HLA-C)⁵³ and mouse (H-2K and H-2D)⁵⁴ code for independent membrane glycoproteins (present on virtually all cells) which are associated with β_2 -microglobulin⁵⁵. The great majority of macromolecules that have been studied^{48,53,54} cap independently, which implies the absence of stable long range ordering of these membrane macromolecules. Moreover, when ligand-induced redistribution is avoided, ultrastructural mapping studies, using ferritin-conjugated antibodies and lectins, generally show a diffuse distribution of antigens and lectin receptors on lymphocytes⁵⁶ and other dissociated cells.

On the other hand, on cells interacting with each other in tissues or in culture, there is in many cases an uneven distribution of plasma membrane proteins. In epithelial cells, certain plasma membrane transport sites⁵⁷ and other membrane enzymes⁵⁸ are restricted to only one surface of the cell, although they are presumably able to diffuse over large areas of the membrane in which they are confined. More severe restriction of movement exists in the case of gap junctions—those points at which cells in tissues or in culture are ionically (electrically) and metabolically coupled. These junctions are seen in freeze fracture microscopy⁵⁹ as polygonal arrays of intramembrane particles; they presumably arise from randomly distributed units in each cell membrane which become highly ordered on cell contact. One of the most striking cases of immobile surface proteins is the acetylcholine receptor of the neuromuscular junction. These molecules are confined to the area of the endplate in innervated striated muscle and probably remain fixed even after denervation⁶⁰. The metabolic turnover of gap junction proteins⁶¹ and junctional acetylcholine receptors⁶² is extremely slow compared with the turnover of most other membrane proteins^{61,63}. This suggests, perhaps, that immobile proteins may be the most stable class of membrane protein.

Although it is unknown how the polarity of epithelial cells is generated, or how junctional acetylcholine receptors are held in place, in principle there are several means by which the distribution and movement of membrane proteins might be controlled. These include the formation of large aggregates of protein which could diffuse only very slowly, as is

the case in the purple membrane of *Halobacterium halobium*, which is a planar crystalline lattice of membrane proteins⁶⁴, and tethering membrane proteins from outside the cell by external fibrous proteins⁶⁵, or by cell-cell contact, as in gap junctions.

A quite different mechanism for controlling the distribution and movement of cell surface proteins became apparent with the discovery of antibody-induced capping of surface antigens. The process of capping, and probably also cell locomotion over a surface, involves the directional movement of cross-linked proteins in the membrane whilst allowing free movement of other proteins. Recent evidence suggests that, during capping, those surface proteins which are moved, are tethered to a cytoplasmic matrix which includes microfilaments and possibly microtubules.

Intracellular fibrillar structures in nucleated cells

The three main types of intracellular fibrillar structure seen by electron microscopy in most nucleated mammalian cells are microtubules, microfilaments and intermediate filaments.

Microtubules⁶⁵, are large, rigid, cylindrical molecules built from a protein subunit called tubulin. Each tubule is composed of 13 parallel 50-Å filaments arranged as a tube of variable length around a hollow 150-Å core. They often form an interconnected cytoskeletal network (through their attachment to centrioles) in the cytoplasm of most mammalian nucleated cells. They are also present in cilia, flagella, and sperm tails where they help produce the motion of those organelles, and in mitotic spindles where they help move chromosomes during mitosis. Cytoplasmic microtubules are usually in a state of rapid assembly and disassembly, processes whose control is still unclear, although Ca^{2+} (ref. 65) and the cyclic nucleotides^{66,67} may be involved. They can be induced to dissociate by pressure and by cold, and their assembly is inhibited by certain plant alkaloids (such as colchicine and vinblastine) which bind with high affinity to tubulin. Evidence suggests that cytoplasmic microtubules may be involved in controlling membrane protein movement and distribution. For example, the binding of concanavalin A (con A) to specific sugar residues on a cell is known to inhibit antibody-induced redistribution of various cell membrane antigens; this inhibition can be relieved by manoeuvres that disrupt microtubules⁶⁸. This observation has been taken to indicate that many different membrane glycoproteins are linked to the microtubule system⁶⁸. As con A is known to bind to a heterogeneous population of membrane glycoproteins, however, it seems possible that it inhibits the movement of otherwise unrestricted lectin receptors by cross-linking them to a subclass of con A-binding glycoproteins which are fixed to the microtubule system⁶⁹. Since microtubules as seen by electron microscopy do not interact directly with the plasma membrane of most mammalian cells, their influence on membrane proteins must be indirect, perhaps through microfilaments.

Microfilaments⁷⁰ are seen in all nucleated cells as fine filamentous structures with a diameter of about 50 Å. They can be decorated with muscle heavy meromyosin (the active head part of myosin)⁷⁰, or with fluorescent anti-actin antibody⁷¹; this, together with studies on isolated microfilaments, shows that they are made of subunits very similar, if not identical, to skeletal muscle actin. In the cell they are associated with cytoplasmic myosin⁷² and tropomyosin⁷³. Microfilaments seem to exist as at least two distinguishable species: "lattice microfilaments", which form a loose network of short, interconnected filaments which may be associated with the cytoplasmic side of plasma membranes and which seem to be sensitive to the drug cytochalasin B, and "sheath" or "stress" microfilaments (also called "actin cables")⁷¹ which exist as bundles of fibres running beneath

the plasma membrane, often parallel to the long axis of fibroblastic cells. These bundles of microfilaments appear to be less sensitive to cytochalasin B (ref. 70). It is likely that both types of microfilaments are contractile, and collectively have an important muscle-like role in cytoplasmic streaming, various forms of plasma membrane movement, in cell shape changes and locomotion. Their possible function in regulating membrane protein mobility is suggested by the demonstration that cytochalasin B (in conditions where its effects on membrane transport are minimised) completely inhibits concanavalin A-induced capping of con A-binding sites on lymphocytes and enables preformed caps to disperse⁶⁸. This suggests that lattice microfilaments have an important role in the capping process, and also in holding the cap at the tail of the cell. There is a hint that, in some circumstances, microfilaments and microtubules may act synergistically in lymphocyte capping; microtubule-disrupting drugs alone do not inhibit capping of immunoglobulin by anti-immunoglobulin antibodies on mouse lymphocytes, and it is only partly inhibited by cytochalasin B (ref. 48), but when cytochalasin B and drugs which disrupt microtubules are used together, they completely inhibit and partly reverse immunoglobulin capping⁶⁹. Although caution is required in interpreting these drug effects, it seems likely that microtubular and microfilament networks can cooperate with each other, the former (possibly together with sheath microfilaments) perhaps providing a structural framework relative to which lattice microfilaments and membrane macromolecules can be displaced.

Intermediate filaments⁷¹ are approximately 100 Å in diameter. In neurones they are known as neurofilaments but similar filaments are found in most mammalian cells, often intermingled with microtubules. Their chemistry and function are unknown, and no specific reagents have been shown to disrupt them. Tonofilaments are of similar diameter and are found attached to the plasma membrane of epithelial cells at specialised sites called desmosomes.

At present, the intense interest in these intracellular fibrous proteins is aimed at understanding their structure and function. One would like to know the nature and extent of their heterogeneity and interactions, how they interact with cell membranes, and the factors that control their orientation within the cell, their assembly and disassembly.

Intramembrane particles in nucleated cell plasma membranes

In nucleated mammalian cells, such as lymphocytes or fibroblasts, freeze fracture electron microscopy reveals intramembrane particles more heterogeneous in size and less densely distributed than in erythrocytes. This greater heterogeneity presumably reflects the large variety of globular proteins in, and thus the greater biochemical complexity of, these membranes. Furthermore, unlike in erythrocytes, most attempts to demonstrate a relationship between the particles and specific cell surface receptors and antigens have failed. Thus, when surface antigens or lectin receptors are capped on lymphocytes⁷², or when lymphocyte surface proteins are covalently labelled with haptenic groups and these are capped with anti-hapten antibody⁷⁶, no equivalent redistribution of the intramembrane particles is seen. Moreover, when lymphocytes or fibroblasts are exposed to high concentrations of glycerol, which reversibly aggregates the particles in these cells⁷⁷, no concomitant aggregation of con A-ferritin-binding sites is seen by electron microscopy (N. B. Gilula, D. Lawson and M.C.R., unpublished). Thus, it seems that the majority of proteins on the surface of these cells that bind antibodies or lectins, or that can be readily haptened, are not associated with the particles.

This inability to identify particles with external receptors and antigens probably results from the existence of two

different classes of surface proteins in nucleated cells. Both may span the plasma membrane, but they are otherwise distinguished in the following ways. One group has an extensive region of the polypeptide and attached oligosaccharide exposed on the outer surface of the cell but little mass in the plane of the bilayer, as in the case of the erythrocyte MN glycoprotein. These surface components would be expected to bind ligands well, but, by themselves, would not be seen in the freeze fracture plane because their intramembrane mass is too small. What happens to such a protein during freeze cleavage? Either one or other region of the polypeptide chain on either side of the membrane pulls out of the ice with which it is in contact, or covalent bonds in the protein are broken. Something must give. For example, it is known that in Semliki Forest virus the spike proteins span the viral membrane⁷⁸, yet no particles are seen in the fracture plane. It seems possible that in this case, and possibly many others, the covalent backbone of that region of the protein inside the membrane has been ruptured. Proteins of the other class are globular, have most of their mass in the plane of the membrane and thus are seen as particles, but do not extend appreciably outside the external surface of the plasma membrane bilayer and are therefore rather inaccessible to ligands. The major erythrocyte protein (of 100,000 daltons, band 3) seems to have these properties; it is remarkably unreactive on its external surface⁷⁹ and would thus be difficult to haptene. In other plasma membranes, hapteneisation of similar globular proteins may also prove to be difficult; an attempt to aggregate them by anti-hapten antibody might therefore be unsuccessful and leave them (and thus the particles) unmoved. It seems likely that this latter class of globular membrane protein would include the many proteins involved in the active and passive transport of small molecules (such as ions, sugars, nucleosides and amino acids) and in the formation of cell junctions.

Most of the globular proteins associated with transmembrane functions are difficult to purify and study biochemically. The great value of the freeze fracture technique is that it shows us the distribution of these proteins in the plane of the membrane with remarkable clarity. What would improve its power enormously would be some general method for relating components, identified either as a band on a gel or by serological methods, to the particles seen in the freeze fracture plane of a membrane. Freeze fracture autoradiography and defining particle subclasses by improved shadowing techniques should prove extremely useful in this respect (D. Branton, personal communication).

Membrane carbohydrate

The proportion of carbohydrate in plasma membranes is never more than 10% by mass and often much less. It is present mainly as oligosaccharide side chains covalently attached to proteins and, to a lesser extent, sphingolipids. Although more than one hundred different monosaccharides are found in nature, only about ten occur in membrane glycoproteins and glycolipids; the principal sugars are galactose, mannose, fucose, galactosamine, glucosamine, glucose and sialic acid⁷⁹, the last usually being terminal and the chief contributor to the net negative surface charge carried by all mammalian cells. Glycolipids and glycoproteins contain similar sugars, except that in glycolipids, mannose is generally absent and glucose is usually found as the first sugar linked to lipid. The oligosaccharide side chains of membrane glycoproteins, and of a small number of glycolipids, can be remarkably complex. Although they are rarely more than 15 sugar residues long, they are usually branched, and can be bonded together in a variety of different linkages⁷⁹. This could give rise to a great range of structures so that, for example, three different sugar

molecules can be arranged into hundreds of different trisaccharides. Although substantial progress has been made in elucidating the sequences of many oligosaccharides bound to lipid or protein, the biological functions of the carbohydrate remain obscure. In both internal and plasma membranes the carbohydrate seems to be located exclusively on the non-cytoplasmic surface^{18,80} and several functions have been proposed to take this restricted location into account.

At one level, carbohydrate is regarded as important for membrane biosynthesis²⁰. It is likely that in nucleated cells all membrane proteins are initially incorporated into the rough endoplasmic reticulum. From there, they diffuse, or are conveyed, to their different membrane locations within the cell—the smooth endoplasmic reticulum, nuclear membrane, Golgi apparatus or plasma membrane. But the detailed mechanism for how these proteins are initially placed in the rough endoplasmic reticulum is unknown; it is often supposed that this is done during synthesis by secretory ribosomes bound to the endoplasmic reticulum, implying that membrane proteins are a special class of secreted protein. The alternative view²⁰ is that membrane proteins are initially synthesised as soluble proteins which dissolve into the endoplasmic reticulum from the cytoplasm. To “lock” these proteins into the membrane, they are glycosylated from the lumen side of the endoplasmic reticulum by a large number of different glycosyl transferases, which are specific for the sugar, acceptor site and the linkage catalysed. This scheme places the onus of anchoring those proteins (which span the membrane) on the carbohydrate. Without this “lock”, the protein could diffuse back into the cytoplasm, a situation which would make it much more difficult for a cell to maintain specific proteins in specific membranes. In bacteria, in which only one membrane (the plasma membrane) exists, no such problem arises and membrane proteins are generally not glycosylated. Once in the rough endoplasmic reticulum, proteins destined for the plasma membrane move to the Golgi complex where more extensive glycosylation occurs; finally, they pinch off the Golgi apparatus in vesicles which fuse with the plasma membrane⁸¹.

But the idea that carbohydrate acts as a “lock” on those proteins which span the membrane does not explain the observed complexity of carbohydrate sequences. A simple oligosaccharide (such as penta-sialic acid) would do as a “lock”, without any need for the wide variety of oligosaccharide chains which exist. One is therefore inclined to look further for additional functions. The potential of complex oligosaccharide labels for the purpose of specific cell-cell recognition has not escaped notice⁸², and it seems possible that they could have such a role. The indirect nature of the genetic control, however, and the resulting variability in the extent of glycosylation, could allow a worrying amount of degeneracy if such a system were used for fine specificity in cell marking. Nevertheless, the consistent differences in oligosaccharide side chains between and among the different glycolipids and glycoproteins indicate the existence of important mechanisms for controlling their glycosylation.

The terms “cell coat” or “glycocalyx” refer to the carbohydrate-rich peripheral zone found at the surface of all mammalian cells, which can be visualised by a variety of stains more or less specific for carbohydrate, such as ruthenium red⁸³. The carbohydrate consists of the oligosaccharide side chains of plasma membrane glycolipids and glycoproteins, including in some cases, adsorbed glycoproteins and mucopolysaccharides (proteoglycans). This high concentration of cell surface carbohydrate must have an important influence on many of the functions of the plasma membrane, but the nature of these influences is poorly understood. The use of highly purified glycosidases and mutant cell clones deficient in certain cell surface monosaccharides⁸⁴ should prove useful in this respect.

Conclusions

All biological membranes, including mammalian plasma membranes, are structurally based on a lipid bilayer. The strongest evidence for this comes from the susceptibility of the membranes to fracture across their mid-planes at low temperature. The bilayer can be visualised as a two-dimensional fluid, with the individual lipid molecules asymmetrically distributed between the two leaflets and able to diffuse in the plane of their own monolayer. Into this lipid matrix, which provides the basic structure of the membrane, specific proteins are inserted asymmetrically and these are responsible for most of the membrane's functional properties. In the case of the plasma membrane, these functions include the receiving and transducing of various extracellular chemical signals (such as hormones, growth factors, neurotransmitters, antigens), cell junction formation (gap, tight, and synaptic), transport of small molecules, endocytosis (pinocytosis and phagocytosis) and exocytosis. Some of the membrane proteins span the bilayer, either as a simply coiled hydrophobic segment (as in the erythrocyte MN glycoprotein) or in a more folded globular state. Most, if not all, of these are glycosylated, having all of the carbohydrate on the non-cytoplasmic side, which probably serves to lock the proteins in place. Since there is no evidence in the erythrocyte (or any other) plasma membrane for a protein which is inserted solely into the outer monolayer of the membrane, it is likely that every membrane glycoprotein spans the bilayer. It seems probable that most of the polypeptides exposed on the cell surface which do not span the membrane (for example, β_2 -microglobulin) are held in place by association with other polypeptides which do so.

In human erythrocytes, the major glycoproteins which span the membrane probably comprise the majority of the intramembrane particles seen in the fracture plane; they do not turn over (if lost, they could not be replaced) and their in-plane mobility is markedly restricted, probably because they are tethered from within by spectrin. In contrast, in nucleated cells, most of the readily detectable surface-exposed plasma membrane proteins seem not to be associated with intramembrane particles, are rapidly turning over and are mobile in the membrane plane; on the other hand, membrane proteins interacting between nucleated cells at sites of specialised intercellular junctions often appear to be associated with particles, are restricted in translational mobility, and turn over comparatively slowly. Little is known about the in-plane mobility or turnover of non-junctional proteins seen as particles. In addition, there is evidence that the cytoplasmic fibrous proteins of nucleated cells, such as microfilaments and microtubules, can control the distribution and translational movement of some membrane proteins.

There are obvious advantages in having a fluid membrane in which molecules can diffuse freely. It may enable membrane enzymes and their lipophilic substrates to come together and interact, and it provides a simple means of distributing proteins and lipids to regions of the membrane remote from where they are inserted, and for dividing membrane components evenly between daughter cells at the time of cell division. In addition, it would be expected to facilitate cell locomotion and membrane fusion, and allow a more adaptable fit between receptors and multivalent ligands or other cells. The alternative of having a frozen and thus rigid lipid bilayer could be dangerous to the cell, as a slight deformation might crack the membrane and lead to the loss of cytoplasmic components. On the other hand, in some circumstances (such as during locomotion), a mechanism for controlling the distribution and mobility of membrane proteins within the fluid milieu is essential. This balance between freedom and control is seen in all things, not least of which is the mammalian plasma membrane.

We thank Drs B. Gomperts and R. D. Kornberg for helpful discussions, and Drs F. Frankel, B. Gomperts, C. Hughes, R. D. Kornberg and A. Raff for commenting on a first draft of the manuscript. The bibliography is incomplete and meant only as a way into the relevant literature; it often refers to reviews and recent examples at the expense of original observations.

- ¹ Oseroff, A. R., Robbins, P. W., and Burger, M. M., *A. Rev. Biochem.*, **42**, 647-682 (1973).
- ² Gorter, E., and Grendel, F., *J. exp. Med.*, **41**, 439-443 (1925).
- ³ Danielli, J. F., and Davson, H., *J. Cell Physiol.*, **5**, 495-510 (1956).
- ⁴ Chapman, D., in *Biological Membranes* (edit. by Chapman, D., and Wallach, D. F. H.), **2**, 91-144 (Academic, New York, 1973).
- ⁵ Kornberg, R. D., and McConnell, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2564-2568 (1971).
- ⁶ Kornberg, R. D., and McConnell, H. M., *Biochemistry*, **10**, 1111-1120 (1971).
- ⁷ Brulet, P., and McConnell, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1451-1455 (1975).
- ⁸ Johnson, L. W., Hughs, M. E., and Zilversmit, D. B., *Biochim. biophys. Acta*, **375**, 176-185 (1975).
- ⁹ Rothman, J. E., and Davidowicz, E. A., *Biochemistry*, **14**, 2809-2816 (1975).
- ¹⁰ Hubbell, W. L., and McConnell, H. M., *J. Am. chem. Soc.*, **93**, 314-326 (1971).
- ¹¹ McConnell, H. M., *Adv. exp. med. Biol.*, **51**, 103-108 (1974).
- ¹² Machtiger, N. A., and Fox, C. F., *A. Rev. Biochem.*, **42**, 575-600 (1973).
- ¹³ Coleman, R., *Biochim. biophys. Acta*, **300**, 1-30 (1973).
- ¹⁴ Hong, K., and Hubbell, W. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2617-2621 (1972).
- ¹⁵ Vanderkooi, G., *Biochim. biophys. Acta*, **344**, 307-345 (1974).
- ¹⁶ Marr, A. G., and Ingraham, J. L., *J. Bact.*, **84**, 1260-1267 (1967).
- ¹⁷ Sinensky, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 522-525 (1974).
- ¹⁸ Steck, T. L., and Dawson, G., *J. biol. Chem.*, **249**, 2135-2142 (1974).
- ¹⁹ Bretscher, M. S., *J. molec. Biol.*, **71**, 523-528 (1972).
- ²⁰ Bretscher, M. S., *Science*, **181**, 622-629 (1973).
- ²¹ Zwaal, R. F. A., Roelofsen, B., and Colley, C. M., *Biochim. biophys. Acta*, **300**, 159-182 (1973).
- ²² Siekevitz, P., *A. Rev. Physiol.*, **34**, 117-140 (1972).
- ²³ Shohet, S. B., *New Engl. J. Med.*, **286**, 257-283 (1972).
- ²⁴ Cuatrecasas, P., *Biochemistry*, **12**, 3558-3566 (1973).
- ²⁵ Van Heyningen, W. E., *Nature*, **249**, 415-417 (1974).
- ²⁶ Singer, S. J., *A. Rev. Biochem.*, **43**, 805-833 (1974).
- ²⁷ Simons, K., Helenius, A., and Garoff, H., *J. molec. Biol.*, **80**, 119-133 (1973).
- ²⁸ Greffrath, S. P., and Reynolds, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3913-3916 (1974).
- ²⁹ Steck, T. L., *J. Cell Biol.*, **62**, 1-14 (1974).
- ³⁰ Juliano, R. L., *Biochim. biophys. Acta*, **300**, 341-378 (1973).
- ³¹ Tomita, M., and Marchesi, V. T., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2964-2968 (1975).
- ³² Clarke, M., *Biochem. biophys. Res. Commun.*, **45**, 1063-1070 (1971).
- ³³ Findlay, J. B. C., *J. biol. Chem.*, **249**, 4398-4403 (1974).
- ³⁴ Cabantchik, Z. I., and Rothstein, A., *J. Membr. Biol.*, **15**, 207-226 (1974).
- ³⁵ Lin, S., and Spudich, J. A., *J. biol. Chem.*, **249**, 5778-5783 (1974).
- ³⁶ Kathan, R. H., and Winkler, R. J., *J. biol. Chem.*, **238**, 21-25 (1963).
- ³⁷ Branton, D., *Phil. Trans. R. Soc.*, **B261**, 133-138 (1971).
- ³⁸ Pinto da Silva, P., Douglas, S. D., and Branton, D., *Nature*, **232**, 194-196 (1971).
- ³⁹ Tillack, T. W., Scott, R. E., and Marchesi, V. T., *J. exp. Med.*, **135**, 1209-1227 (1972).
- ⁴⁰ Pinto da Silva, P., and Nicolson, G. L., *Biochim. biophys. Acta*, **363**, 311-314 (1974).
- ⁴¹ Peters, R., Peters, J., Tews, K. H., and Bähr, W., *Biochim. biophys. Acta*, **367**, 282-294 (1974).
- ⁴² Elgsaeter, A., and Branton, D., *J. Cell Biol.*, **63**, 1018-1030 (1974).
- ⁴³ Brown, J. C., and Hunt, R. C., *J. molec. Biol.*, **97**, 413-422 (1975).
- ⁴⁴ Hynes, R. O., *Cell*, **1**, 147-156 (1974).
- ⁴⁵ Cone, R. A., *Nature new Biol.*, **236**, 39-43 (1972).
- ⁴⁶ Blasie, J. K., and Worthington, C. R., *J. molec. Biol.*, **39**, 417-439 (1969).
- ⁴⁷ Frye, L. D., and Edidin, M., *J. Cell Sci.*, **7**, 319-335 (1970).
- ⁴⁸ Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225-229 (1971).
- ⁴⁹ de Petris, S., and Raff, M. C., *Nature new Biol.*, **241**, 257-259 (1973).
- ⁵⁰ de Petris, S., and Raff, M. C., *Eur. J. Immun.*, **2**, 523-535 (1972).
- ⁵¹ Lamm, M. E., Boyse, E. A., Old, L. J., Lisowska-Bernstein, B., and Stocker, E., *J. Immun.*, **101**, 99-103 (1968).
- ⁵² Gavin, J. R., Roth, J., Neville, D. M., De Meyts, P., and Buell, D. N., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 84-88 (1974).
- ⁵³ Bernoco, B. S., Cullen, S., Scudeller, G., Trinchieri, G., and Ceppellini, R., in *Histocompatibility Testing 1972* (edit. by Dausset, J.), 527-539 (Munksgaard, Copenhagen 1973).
- ⁵⁴ Neauport-Sautes, C., Lilly, F., Silvestre, D., and Kourilsky, F. M., *J. exp. Med.*, **137**, 511-526 (1973).
- ⁵⁵ Poulik, M. D., Bernoco, M., Bernoco, D., and Ceppellini, R., *Science*, **182**, 1352-1355 (1973).
- ⁵⁶ de Petris, S., and Raff, M. C., *Eur. J. Immun.*, **4**, 130-137 (1974).
- ⁵⁷ Sheffield, J. B., and Emmelot, P., *Expl Cell Res.*, **71**, 97-105 (1972).
- ⁵⁸ Stevenson, F. K., *Biochim. biophys. Acta*, **311**, 409-416 (1973).
- ⁵⁹ Gilula, N. B., in *Cell Communication* (edit. by Cox, R. P.), 1-29, (Wiley, New York, 1974).
- ⁶⁰ Fambrough, D. M., in *Neurochemistry of Cholinergic Receptors*, (edit. by de Robertis, E., and Schacht, J.), 85-105, (Raven, New York, 1974).
- ⁶¹ Gurd, J. W., and Evans, W. H., *Eur. J. Biochem.*, **36**, 273-274 (1973).
- ⁶² Hartzell, H. C., and Fambrough, D. M., *Devl Biol.*, **30**, 153-165 (1973).
- ⁶³ Warren, L., and Glick, M. C., *J. Cell Biol.*, **37**, 729-746 (1968).
- ⁶⁴ Blaurock, A. E., and Stockenius, W., *Nature new Biol.*, **233**, 152-155 (1971).
- ⁶⁵ Olmsted, J. B., and Borisy, G. G., *A. Rev. Biochem.*, **42**, 507-540 (1973).
- ⁶⁶ Gillespie, E., in *Cyclic AMP, Cell Growth and the Immune Response* (edit. by Braun, W., Lichtenstein, M., and Parker, C. W.), 317-325, (Springer, New York, 1974).
- ⁶⁷ Oliver, J. M., Zurier, R. B., and Berlin, R. D., *Nature*, **253**, 471-473 (1975).
- ⁶⁸ Edelman, G. M., Yahara, I., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1442-1446 (1973).
- ⁶⁹ de Petris, S., *J. Cell Biol.*, **65**, 123-146 (1975).
- ⁷⁰ Wessells, N. K., Spooner, B. S., and Luduena, M. A., in *Locomotion of Tissue Cells* (Ciba Foundation Symposium 14, new series), 53-76 (Elsevier Excerpta Medica, North-Holland, Amsterdam, 1973).
- ⁷¹ Lazarides, E., and Weber, K., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2268-2272 (1974).
- ⁷² Weber, K., and Groeschel-Stewart, U., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4561-4564 (1974).
- ⁷³ Lazarides, E., *J. Cell Biol.*, **65**, 549-562 (1975).
- ⁷⁴ Schmitt, F. O., and Samson, F. E., in *Neurosci. Res. Prog. Bull.*, **6**, 113-219 (1968).
- ⁷⁵ Karnovsky, M. J., and Unanue, E. R., *Fedn Proc.*, **32**, 55-59 (1973).
- ⁷⁶ Wofsy, L., in *The Immune System, Genes, Receptors and Signals* (edit. by Sercarz, E. E., Williamson, A. R., and Fox, C. F.), 259-270, (Academic, New York, 1974).
- ⁷⁷ McIntyre, J. A., Gilula, N. B., and Karnovsky, M. J., *J. Cell Biol.*, **60**, 192-203 (1974).
- ⁷⁸ Garoff, H., and Simons, K., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3988-3992 (1974).
- ⁷⁹ Hughes, R. C., *Prog. Biophys. molec. Biol.*, **26**, 189-268 (1973).
- ⁸⁰ Hirano, H., Parkhouse, B., Nicolson, G. L., Lennox, E. S., and Singer, S. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2945-2949 (1972).
- ⁸¹ Palade, G., in *Subcellular Particles* (edit. by Hayashi, T.), 64-83 (Ronald, New York, 1959).
- ⁸² Roth, S., McGuire, E. J., and Roseman, S., *J. Cell Biol.*, **51**, 536-547 (1971).
- ⁸³ Rambourg, A., and LeBlond, C. P., *J. Cell Biol.*, **32**, 27-53 (1967).
- ⁸⁴ Gottlieb, C., Skinner, S. A. M., and Kornfeld, S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1078-1082 (1974).

articles

Mapping of the G₁ phase of a mammalian cell cycle

P. M. Naha, A. L. Meyer & Kathleen Hewitt

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, UK

Temperature-sensitive variant clones of BALB/3T3 cells were put on a linear time scale map by measuring their time of entry into S phase; these clones seemed to accumulate a unique non-histone protein at the restricted temperature.

THERE is an extensive literature on the biochemistry and control mechanisms of the four phases of the cell cycle¹ (for reviews see refs 2 and 3); but little is known of the

biochemical events in G₁ (between completion of mitosis and onset of DNA synthesis) and G₂ (between completion of DNA synthesis and mitosis). The production of RNA and proteins involved in the initiation, progress and termination of DNA synthesis are some of the main events attributed to G₁ and G₂ (refs 2 and 3); however, most of the evidence was indirect since selective inhibitory agents (for example, dactinomycin) were used. Other investigators⁴ have referred to the resting (in respect of proliferation) cells as being in G_n, distinct from G₁, which can be stimulated to divide by an appropriate stimulus⁵. Such non-growth states can be achieved experimentally and attention

has focused recently on cells so arrested in G_1 as potential "target cells" for carcinogenic transformation^{6,7}. It has also been suggested that there are some rate controlling signals in G_1 itself and at the G_1/S boundary which have regulatory functions⁸⁻¹⁰. We have mapped G_1 of a mammalian cell cycle using temperature-sensitive variant cell lines. There have been several reports of variant clones defective in G_1 (refs 11-15), but for a comprehensive study a linear time scale map is essential, and this article presents data on such a map.

An aneuploid clone (A31) of BALB/3T3 (ref. 16) was obtained from Imperial Cancer Research Fund Laboratories, London. The culture was maintained in L-15 medium¹⁷ with 10% foetal bovine serum (FBS), penicillin (100 U ml^{-1}) and streptomycin ($50 \mu\text{g ml}^{-1}$) at 33°C . Temperature-sensitive variant clones were maintained in similar conditions. A preliminary study of the generation time of wild-type BALB/3T3 cells showed that between 33°C and 38°C the cells doubled at approximately the same rate ($21 \pm 0.5 \text{ h}$ and $20 \pm 0.75 \text{ h}$, respectively). Replicate cell cultures plated at 1,000 per Falcon tissue culture plates ($60 \times 15 \text{ mm}$) at 38°C were exposed to doses of N-methyl-N'-nitrosoguanidine (MNNG) (Aldrich, $0.5 \mu\text{g ml}^{-1}$) every 90 min for 9 h after plating. This was done to catch cells at different stages of G_1 in an asynchronous population. After 2 h of treatment with MNNG (lethality less than 50%) cells were exposed to either $2.5 \times 10^{-4} \text{ M}$ 5-bromo-2-deoxyuridine (BUDR, Sigma)¹⁸ or $6\text{-}^3\text{H}$ -thymidine¹⁹ ($5 \mu\text{Ci}$

the temperature-sensitive cells were defective we compared the cell cycles of wild-type BALB/3T3 and temperature-sensitive variants at 33°C and 38°C . In general we followed Baserga's²⁰ method. We counted labelled cells in mitosis in 200 metaphase plates of each sample. The labelled metaphases represented cells that had gone through G_2 , mitosis, G_1 , S, second G_2 and mitosis after a pulse label. The average generation time was estimated from the difference between 50% points on the two ascending limbs of the biphasic curve^{20,21}. Mitotic index was calculated from the same preparations by counting the average number of cells in mitosis in 50 microscopic fields. The data for two temperature-sensitive clones are presented here for comparison with the parental cell line. The division cycle of cells showed (Fig. 1a-c) that the duration of one cell cycle in wild-type BALB/3T3 (Fig. 1a) was approximately 20.5 h at both 33°C and 38°C , but the distribution of the four phases differed slightly. At 38°C , the durations of phases were G_1+M , 6.5 h; S, 10.5 h; and G_2 , 3.5 h, whereas at 33°C they were G_1+M , 8.5 h; S, 9.5 h; and G_2 , 2.5 h. In the variant clones A8 (Fig. 1b) and A83 (Fig. 1c), however, S seemed to be slightly longer at 33°C , but at 38°C both clones failed to reach S. Labelled cells in mitosis did not exceed 50% in the variant clones at 38°C . This was true for all eight variant clones.

Mitotic indices counted from the same preparations showed (Fig. 1a-c) that at 33°C the variant clones A8 and A83, behaved very similarly to the parental cell line,

Table 1 Colony-forming ability of BALB/3T3 and temperature-sensitive variants at 33°C and 38°C after 7-10 d of incubation

Clones	5×10^2 cells		No. of input cells per 25 cm^2 Falcon tissue culture flasks		5×10^3 cells		1×10^4 cells	
	33°C	38°C	33°C	38°C	33°C	38°C	33°C	38°C
Wild type	56 ± 3	61 ± 7	82 ± 7	116 ± 4	c	c	c	c
A8	29 ± 3.3	0	71 ± 5	0	c	26 ± 8	c	73 ± 11
A83	33 ± 4	0	59 ± 10	0	c	32 ± 13	c	92 ± 14
A90	30 ± 6	0	58 ± 12	0	c	45 ± 18	c	108 ± 22
A92	23 ± 1	0	63 ± 2.8	0	c	92 ± 15	c	c
A122	59 ± 5	0	117 ± 4	0	c	97 ± 7	c	c
A123	63 ± 8	0	123 ± 12	0	c	58 ± 12	c	138 ± 8
B23	18 ± 4.6	0	40 ± 5.5	0	c	106 ± 10	c	c

To determine colony-forming ability, trypsinised cells were plated in 25 cm^2 Falcon tissue culture flasks at a density of 10,000, 5,000, 1,000 and 500 per flask in L-15 medium. Replicate cultures were incubated at 33°C and 38°C for 7-10 d. Half the medium was replaced every 3 d. Finally, the cultures were stained in Giemsa. c, Confluent culture (> 250 colonies).

ml^{-1} , 32 Ci mmol^{-1}) for 2 h in a prewarmed medium at 38°C to kill the DNA-synthesising cells selectively. The culture plates were then washed in normal growth medium (L-15) with 10% FBS and incubated at 33°C until discrete colonies were visible. Half the medium was replaced every 3 d. Isolated clones were purified further from single cell colonies through two successive reclonings. Forty-five temperature-sensitive clones were isolated after preliminary selection in this way. Of these, eight were studied because they were relatively the least 'leaky'—A8, A69, A83, A90, A92, A122, A123 and B23.

These variant clones differed (4-12%) in their ability to form colonies at 33°C when plated at 1,000 and 500 cells per flask, but almost no colony grew in any of them at 38°C at these densities (Table 1). Compared with the variant cells, the parental cell line had more colonies growing at 38°C (11.6%) than at 33°C (8.2%). At greater densities (5,000 and above) variant clones would grow and appeared more and more leaky at the restricted temperature (38°C). Temperature shift-down after 10 h at 38°C revealed no significant decrease in colony formation in any temperature-sensitive variant. This suggested that the temperature-sensitive defects were at least partly reversible, but were irreversibly impaired during prolonged incubation at 38°C . To pinpoint the phase of the cell cycle when

the mitotic index increasing from 1-2% to about 4.8%. At 38°C , however, mitotic indices were significantly different. The parental culture and clone A8 had mitotic indices more or less identical to those at 33°C ; in clone A83, on the other hand, the mitotic index rose from 1.6% to 48.8% between 12 and 24 h at 38°C . The latter was at first thought to indicate cells arrested in mitosis, similar to the situation with colchicine treatment, but an analysis of the cells at different stages of mitosis (prophase : metaphase : anaphase : telophase) was more or less similar between 12 and 24 h (1.6: 5.6: 1.0: 1.5 respectively). If anything there was an accumulation of cells in anaphase and telophase during the later stages of incubation of A83 at 38°C . This may represent a slowing down of 'transit' through all phases of mitosis. We checked this by testing the viability of the cells arrested at 38°C by means of temperature shift-down (from 38°C to 33°C). We found that the longer the cultures were incubated at 38°C , the larger the number of cells entering S phase, though they were delayed. This indicated that many A83 cells arrested at 38°C remained viable for the next 10-12 h. The variants were mapped by timing the start of DNA synthesis. For this purpose, 1×10^5 cells per ml were seeded in Leighton tubes on coverslips in 1 ml volumes as before. The culture tubes were incubated for one generation time (20 h) at 33°C ; thereafter the cultures

were transferred to 38 °C except for one set of controls. Replicate culture tubes (in triplicate) were incubated at 38 °C for 2, 4, 6, 8 and 10 h and transferred back to 33 °C for 8, 6, 4, 2 and 0 h, respectively. From the 10th hour,

culture tubes from each set of transfers were pulse labelled for 10 min with $6\text{-}^3\text{H}$ -thymidine ($5\text{ }\mu\text{Ci ml}^{-1}$, 32 mCi mmol^{-1}) at 33 °C every hour for 12 h. Preliminary studies of the cell cycle of the parental line (Fig. 1) showed that the cells

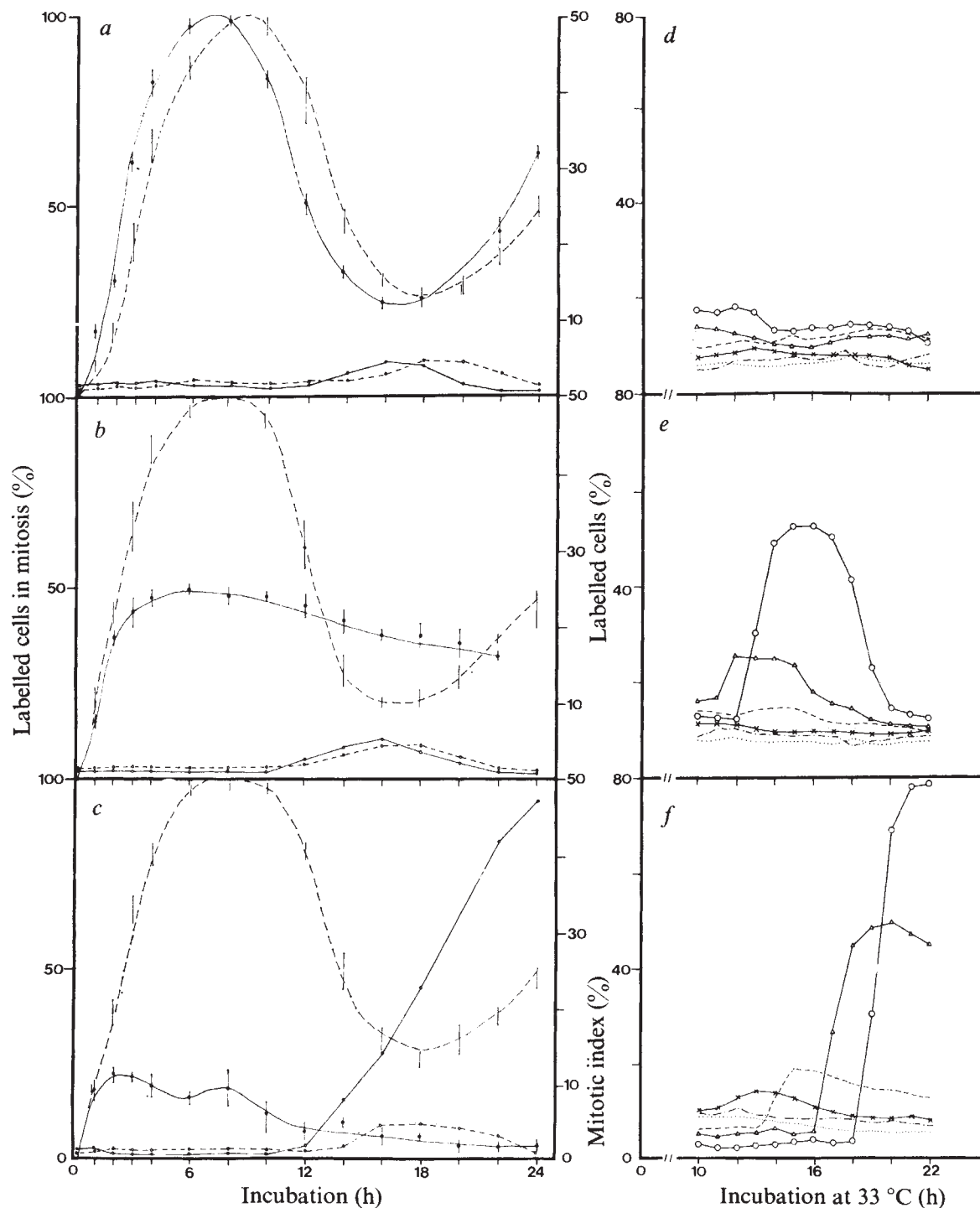


Fig. 1 *a-c*, Represent the cell cycle and mitotic indices of BALB/3T3 (*a*), and the temperature-sensitive variants A8 (*b*) and A83 (*c*). ---, 33 °C; —, 38 °C. *d-f*, Represent the time of entry into S phase in the respective cultures (BALB/3T3, *d*; A8, *e*; A83, *f*) during temperature shift-down after incubation at 38 °C for different times. ···, Control at 33 °C; - - -, 2 h at 38 °C; ×, 4 h at 38 °C; - - - - -, 6 h at 38 °C; Δ, 8 h at 38 °C; ○, 10 h at 38 °C. Cells at a density of $1 \times 10^5\text{ ml}^{-1}$ were planted on coverslips in Leighton tubes in 1-ml volumes and incubated for 36 h at 33 °C to enable the cells to reach the log phase of growth. Cells were then exposed to $6\text{-}^3\text{H}$ -thymidine at a concentration of $1\text{ }\mu\text{Ci ml}^{-1}$ (5 Ci mmol^{-1}) for 10 min and washed in Hanks' balanced salt solution (BSS) containing cold thymidine ($5\text{ }\mu\text{g ml}^{-1}$) to dilute the remaining radioactivity in the cells. Finally, the cells were placed in L-15 medium containing cold thymidine ($5\text{ }\mu\text{g ml}^{-1}$); replicate tubes were incubated at 33 °C and 38 °C. At hourly or two-hourly intervals one set of tubes was fixed, autoradiographed and stained by the following method. After removing the medium, cells were treated with a hypotonic solution of Hanks' BSS in water (1:4) for 5 min, fixed for 5 min in acetic acid: ethanol (1:3) diluted in the hypotonic solution, and then fixed for a further 10 min in undiluted fixative. The coverslips were air dried and mounted in glass slides with the cells on the upper side. The slides were then dipped in Ilford L-4 liquid emulsion and exposed for 2 weeks in the dark at -20 °C. After 2 weeks the slides were developed in D19 developer, dried and stained in 4% buffered Giemsa.

entered S between 8.5 and 10 h. The design of our experiments was expected to enable the parental cell line to enter without delay; but a fractional population with a defect in G_1 in the variant cell line would be delayed in its biochemical processes and would thus enter S late. The time a variant culture was delayed in entering S was calculated to be the time (map distance) from the S phase with respect

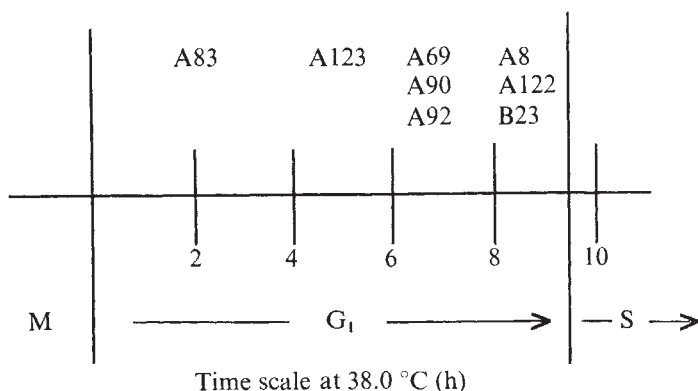


Fig. 2 Tentative map of the G_1 phase of the cell cycle of temperature-sensitive clones of BALB/3T3 cells, measured with respect to the time of the block and entry into S phase.

to the parental culture. This map of G_1 variants is strictly a measure of their time of entry into S; it is not a complementation or genetic map.

The coverslips from each tube after exposure to radioactivity were washed, fixed and autoradiographed as in the cell cycle experiment. The percentage of labelled cells was counted from 50 microscopic fields; cells in active DNA synthesis were counted for 12 h. The results showed (Fig. 1d-f) that in the wild type parental culture (Fig. 1d) there was no G_1 accumulation of cells entering S during successive transfers from 38 °C; if anything, the percentage of labelled cells was slightly higher in the last two transfers at 8 h (9.6%) and at 10 h (14.6%), indicating more cells already entering S at 38 °C. The number of labelled cells, however, decreased as the culture progressed through the growth cycle at 33 °C. The variant clones A8 (Fig. 1e) and A83 (Fig. 1f), on the other hand, had presumably accumulated cells in G_1 during longer periods of incubation at 38 °C so that the cells were delayed in entering S for different times; this was reflected in the larger number of labelled cells appearing in later transfers. In the variant clone A8, the delay in entering the S phase and consequent accumulation of labelled cells appeared between 8 and 10 h incubation at 38 °C, whereas in clone A83 the delay and accumulation of cells entering S were first observed between 2 and 4 h incubation at 38 °C. It is interesting to compare the corresponding increase in the percentage of labelled cells in the variant clones during longer periods of incubation at 38 °C. In clone A8, the number of labelled cells increased from 16.6% to 25.8% after 8 h of incubation at 38 °C (Fig. 1e); but after 10 h at 38 °C the number of labelled cells increased from 12.0% to 52.2%, though entry into S was delayed by 2 h in the latter case. The same appeared to be true for A83. After 4 h of incubation at 38 °C (Fig. 1f) cells started to accumulate in G_1 , denoted by a corresponding delay in entering S at 33 °C. The percentage of labelled cells among those transferred after 8 h increased from 5.9% to 58.4%; after 10 h at 38 °C it increased from 3.8% to 78.4%. These results indicated that the variant cultures attained 'partial synchrony' in entering S phase because they had longer periods of incubation at the res-

tricted temperature (38 °C); the cells were presumed to be arrested in G_1 .

The 'partial synchrony' was soon lost after the cells were shifted to 33 °C. In both A8 and A83, 'synchronised' populations began to lose synchrony within 3-4 h at 33 °C; the percentage of labelled cells decreased sharply instead of forming a plateau. Since the measured time for S phase was between 10 and 12 h at 33 °C, the synchrony was probably lost halfway into the S phase in these variant cell lines.

The duration of the S phase in the parental and variant (A8, A83) cultures being about 10 h at 33 °C (half the generation time for each cell), at least 50% of cells would be expected to be labelled with ^3H -thymidine at any time in the control cultures, but we observed only 10-20% labelling of cells at 33 °C. This was thought to be due to either (1) lower efficiency of plating (<50%) in these cells, or (2) many cells not actively cycling in these early log phase cultures incubated for 20 h at 33 °C. This series of experiments was conducted for each of the other six variant clones. On the basis of their time of entry in S, the 'mutant' cells were tentatively put on a linear time scale map (Fig. 2). (The final assignment of these 'mutant' clones on a

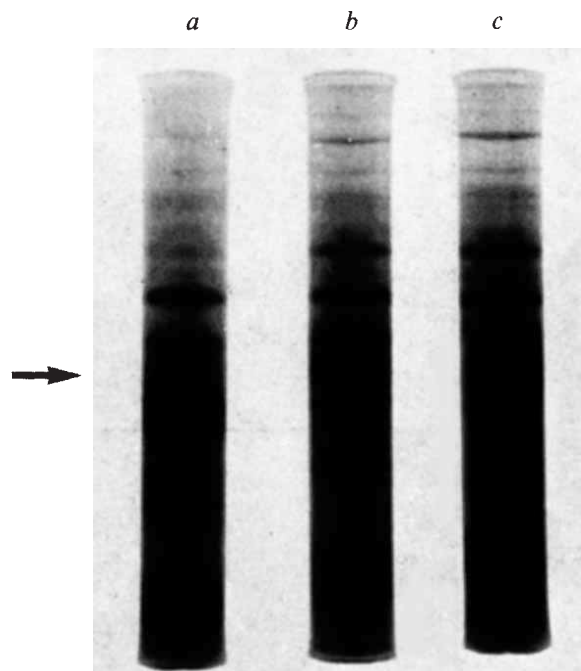


Fig. 3 Polyacrylamide gel electrophoresis of 100 μg of nuclear proteins of cultures, at 38 °C, of parental cell line of BALB/3T3 (a), clone A8 (b) and clone A83 (c). Cells at a density of $1 \times 10^{-5} \text{ ml}^{-1}$ in 30 ml volumes were plated in 16-ounce glass bottles and incubated for 16 h at 38 °C. Cultures were collected from the bottles after two washes with cold phosphate-buffered saline (PBS) in 1 ml of 1% Nonidet P40. Cells were then washed with PBS and frozen. Nuclear proteins were prepared by the methods described in Fig. 4. Gels were stained with 2% naphthalene black.

genetic map awaits completion of complementation tests now in progress.)

We analysed the nuclear proteins in the two variant clones (A8, A83) and the parental clone, grown for 16 h at the restricted temperature (38 °C) to identify possible biochemical alteration in the nuclei of the variants at the restricted temperature. Nuclear proteins were prepared as before²². Equal amounts (100 μg in 0.2 ml) were run on 6% acrylamide disc gels with sodium dodecyl sulphate in a continuous system²². Stained preparations of these gels revealed

(Fig. 3) a unique protein in both variant clones (Fig. 3). On the other hand, several low molecular weight proteins at the bottom of the gel in the parental type were absent from the variant clones. There was, however, similarity in the electrophoretic profiles of the nuclear proteins in the two variant clones which are widely separated in our time scale map of G_1 .

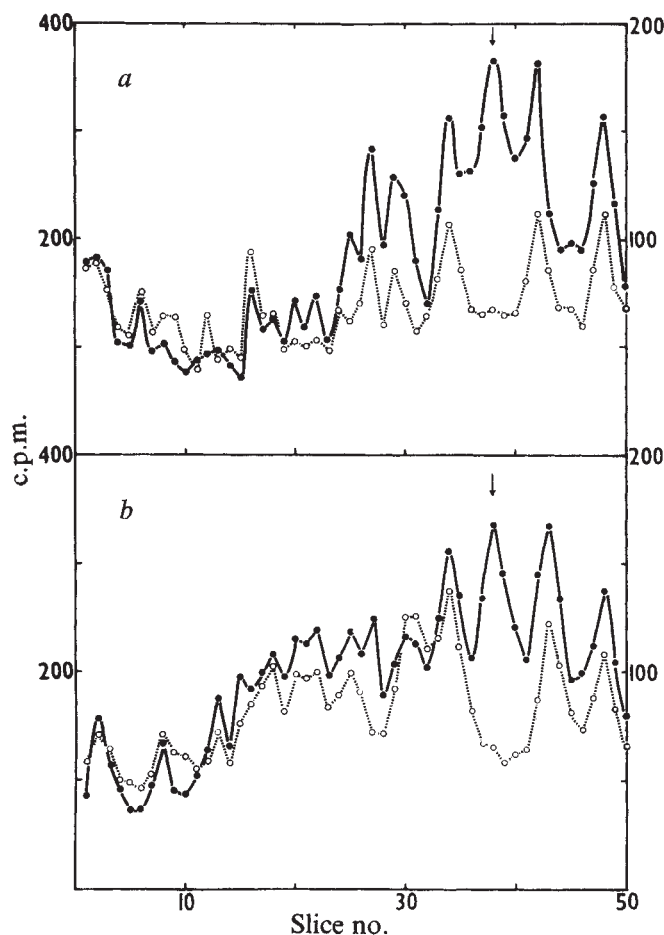


Fig. 4 *a*, Distribution of ^3H -tryptophan (●) and ^{14}C -methionine (○) in 100 µg of nuclear protein of clone A83. Cells were incubated for 16 h at 38 °C and double labelled with radioactive amino acids for 1 h at 38 °C. *b*, Distribution of ^3H -tryptophan (●) and ^{14}C -tryptophan (○) in coelectrophoresis of 100 µg of nuclear proteins of clone A83 grown at 38 °C and 33 °C respectively for 16 h and exposed to radioactive medium for 1 h at their respective temperatures. Quantitation of the nuclear proteins was performed as follows. Cells at a density of $1 \times 10^5 \text{ ml}^{-1}$ in 30 ml volumes were plated in 16-ounce glass bottles and incubated for 16 h at 38 °C. The cells were then washed with Hanks' BSS and exposed to Hanks' BSS with L-methionine-methyl- ^{14}C ($10 \mu\text{g ml}^{-1}$, 56 mCi mmol $^{-1}$), or L-(methylene- ^{14}C)-tryptophan ($10 \mu\text{g ml}^{-1}$, 2 Ci mmol $^{-1}$), and L-tryptophan- ^3H -G ($24 \mu\text{g ml}^{-1}$, 5 Ci mmol $^{-1}$) for 1 h at 38 °C. Cultures were collected from the bottles after two washes, with cold PBS, in 1 ml of 1% Nonidet P40. Cells were then washed with PBS and frozen. Frozen cell pellets were washed and resuspended in rabbit reticulocyte standard buffer (RSB, pH 7.4) and allowed to swell for 10 min at 2 °C. The cells were homogenised on a Dounce homogeniser, using 10–15 strokes. In these conditions, 95–100% of cells released intact nuclei and cytoplasm. Nuclei were centrifuged at 500g for 15 min and washed with RSB. The nuclear pellets were resuspended in RSB (10^7 nuclei ml $^{-1}$) and disrupted by sonication for 5 min at full power in a Soniprobe Type 7530A sonic oscillator. The nuclear fraction (100 µg in 0.2 ml) was first reduced by treating it with 0.5 ml of a mixture of 10 M urea, 4% SDS and 4% mercaptoethanol and heating at 100 °C for 3–5 min. The sample was run on 6% acrylamide gels with SDS in a continuous system. The distribution of radioactive proteins in the gels was determined by slicing the gel into 1-mm thick sections, dissolving it in 0.5 ml of H_2O_2 at 60 °C and counting in toluene scintillation fluid (toluene-Triton, 1:1).

For a qualitative and quantitative estimation of the different proteins in the gels, the variant A83 cells were labelled and the radioactivity in the nuclear proteins was analysed. The distributions of radioactive proteins, doubly labelled at the restricted temperature with ^{14}C -methionine and ^3H -tryptophan showed (Fig. 4*a*) a high rate of incorporation of tryptophan in the band indicated. Since tryptophan is incorporated selectively into non-histone proteins, we presume that this fraction is a non-histone protein. To acquire further evidence that this protein accumulated only at the restricted temperature equal amounts of nuclear proteins (100 µg) of ^3H -tryptophan-labelled A83 cells at 38 °C and ^{14}C -tryptophan-labelled A83 cells at 33 °C were run together; they did not band in the same molecular weight region (Fig. 4*b*). Appearance of an identical non-histone protein in the two variant clones, A8 and A83, defective in G_1 suggests that these variant clones were accumulating a protein that was not involved in turnover at 38 °C. Isolation and characterisation of this protein has now been undertaken.

Our results show that temperature-sensitive cell lines could provide a useful tool for study of the control of the cell cycle in mammalian cell systems, particularly in the identification of endogenous components necessary for the regulation of cell growth. There are increasing indications that differentiation and carcinogenesis are related to regulatory changes in a cell^{23,24} which are possibly determined during G_1 . A tendency for different mammalian cell types to have G_2 and M of similar durations but a G_1 of widely varying durations has been offered repeatedly as evidence that controlling events occur in G_1 (refs 20, 25 and 26). A significant conclusion from our experiments is that some of the events controlled in G_1 are apparently sequential with respect to their biochemical events. Arresting any of these events delayed the subsequent processes.

The molecular mechanism involved in the alterations of a particular enzymatic function in the temperature-sensitive cells is not well understood. Whether the variant clones in a conditionally defective state (at the restricted temperature) make an altered enzymatically inactive protein ("missense") or no protein at all ("nonsense") has yet to be resolved. To what extent these structurally altered proteins could be corrected, and the time it would take for the correct resolution might to a certain extent jeopardise a linear time scale map over short sequences, but over long sequences such a map would probably provide a good foundation for the study of functions controlled in G_1 .

This work was supported by grants from the Medical Research Council and the Cancer Research Campaign.

Received June 19; accepted October 1, 1975.

- Howard, A., and Pelc, S. R., *Hereditas*, Suppl. 6, 261–273 (1953).
- Baserga, R., *Cell Tissue Kinet.*, 1, 167–191 (1968).
- Mitchison, J. M., *The Biology of the Cell Cycle* (Cambridge University Press, London, 1971).
- Lajtha, L. G., *J. cell. comp. Physiol.*, Suppl. 1, 62, 143–144 (1963).
- Pardee, A. B., *Proc. natn. Acad. Sci. U.S.A.*, 71, 1286–1290 (1974).
- Bertram, J. S., and Heidelberger, C., *Cancer Res.*, 34, 526–537 (1974).
- Naha, P. M., *Br. J. Cancer*, 31, 338–347 (1975).
- Rao, P. N., and Johnson, R. T., *Nature*, 225, 159–164 (1970).
- Marshall Graves, J. E., *Expl Cell Res.*, 72, 393–403 (1972).
- Mueller, G. C., *The Cell Cycle and Cancer* (edit. by Baserga, R.), 269–307 (Dekker, New York, 1971).
- Naha, P. M., *Nature new Biol.*, 241, 13–14 (1973).
- Roscoe, D. H., Robinson, H., and Carbonell, A. W., *J. Cell Physiol.*, 82, 333–338 (1973).
- Smith, B. J., and Wigglesworth, N. M., *J. Cell Physiol.*, 82, 339–348 (1973).
- Liskay, R. M., *J. Cell Physiol.*, 84, 49–55 (1974).
- Burstein, S. J., Meiss, H. K., and Basilio, C., *J. Cell Physiol.*, 84, 397–407 (1974).
- Aaronson, S. A., and Todaro, T. J., *Science*, 162, 1024–1026 (1968).
- Leibovitz, A., *Am. J. Hyg.*, 78, 173–180 (1963).
- Naha, P. M., *Nature*, 223, 1380–1381 (1969).
- Thompson, L. H., et al., *J. Cell Physiol.*, 78, 431–440 (1971).
- Baserga, R., *Cancer Res.*, 25, 581–607 (1965).
- Maciera-Coeelho, A., Ponter, J., and Philipson, L., *Expl Cell Res.*, 42, 673–684 (1966).
- Naha, P. M., *Br. J. Cancer*, 31, 590–597 (1975).
- Greenstein, J. P., *Biochemistry of Cancer*, Second ed. (Academic, New York, 1954).
- Paul, J., and Hickey, I., *J. clin. Path.*, 27, Suppl. 7, 4–10 (1974).
- Prescott, D. M., *Cancer Res.*, 28, 1815–1816 (1968).
- Epifanova, O. I., and Tershikh, V. V., *Cell Tissue Kinet.*, 2, 75–89 (1969).

Nuclease that preferentially inactivates DNA containing mismatched bases

A. Ahmad

Max-Planck-Institut für Molekulare Genetik, Innestrasse 63-73, 1 Berlin 33 (Dahlem), West Germany

W. K. Holloman* & R. Holliday

National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

Heteroduplex and homoduplex DNA molecules of bacteriophage SPPI were treated with DNase I, an enzyme of Ustilago maydis implicated in genetic recombination. A transfection assay with Bacillus subtilis demonstrates that the heteroduplexes are preferentially inactivated.

It is widely accepted that hybrid or heteroduplex DNA is an essential intermediate in genetic recombination¹⁻⁵. In heterozygous crosses, the inclusion of a genetic marker within a hybrid DNA segment will result in the formation of a mismatched base pair. It was suggested that gene conversion (non-reciprocal allelic recombination) could be due to the repair of such mismatched bases^{6,7}. Genetic evidence for such repair with artificially made heteroduplexes has subsequently been obtained in transfection experiments with bacteriophage SPPI of *B. subtilis*⁸, in phage λ ^{9,10}, as well as in transformation with *Pneumococcus*¹¹ and studies on gene conversion and post-meiotic segregation in *Ascobolus*¹². An initial step in the repair of mismatched bases is presumed to involve an enzyme that cleaves one strand of the DNA at or near the mismatched region, analogous to the initial step in pyrimidine dimer excision repair.

A search for such an enzyme in the fungus *Ustilago maydis* was initiated by the isolation of DNase deficient mutants, which were also defective in the process of gene conversion^{13,14}. The deficiency of gene conversion was shown to be associated with a low level of a nuclease, DNase I¹⁵. We show here that this enzyme preferentially inactivates heteroduplex molecules containing a single mismatched base pair or a deletion, as well as molecules containing pyrimidine dimers.

Ustilago nuclease and SPPI transfection

The enzyme has been purified 8,600-fold from wild-type extracts and has been fully characterised¹⁴⁻¹⁶. Like the related nucleases of *Neurospora crassa*¹⁷ and *Aspergillus nidulans*¹⁸, it is most active on single-stranded DNA substrates. Although it does not degrade native DNA to acid soluble products, alkaline sucrose gradient analysis showed that single-strand nicks are introduced in such molecules. Previous irradiation of the substrate with ultraviolet light increases the number of nicks introduced, which indicates that the enzyme recognises minor distortions in DNA. This activity on native DNA is not due to a second contaminating nuclease. The enzyme is unusual in having no activity on circular DNA, whether single or double stranded. Free ends of DNA molecules are therefore essential for its activity.

Native DNA from phage SPPI is taken up by competent cells of *B. subtilis*, which later produce a burst of phage particles¹⁹. The strands of SPPI DNA after complexing with RNA can readily be separated in CsCl density gradients. Separated complementary strands obtained from mutant and wild-type phages can be reannealed to form biologically active homoduplex and heteroduplex molecules^{8,20}. It is known (personal communication from H. C. Spatz and T. A. Trautner), that a single-strand

nick inactivates the molecule in the transfection assay. The availability of defined homoduplex and heteroduplex molecules provides a very sensitive substrate for any enzyme which specifically attacks heteroduplex molecules.

Hydroxylamine-induced (G-C → A-T) plaque-type mutants have been isolated in SPPI phage. Most of the experiments involve one of these, mutant 161. The mismatches in heteroduplex DNA therefore involve single bases, the pairs being either adenine-cytosine or guanine-thymine. Many deletion mutants have been isolated. We have used one, 4307, which lacks up to 3% of the genome, as judged by its buoyant density and its sensitivity to citrate inactivation (personal communication from G. Klotz and T. A. Trautner).

Inactivation of hetero- and homoduplex DNA

The two heteroduplexes, two homoduplexes and native DNA were exposed to *Ustilago* nuclease for 15 min under standard assay conditions (see legend to Table 1). Competent cells were added and incubated for 30 min at 37 °C. The cells were then diluted and plated to score the number of infective centres.

The difference between the inactivation of homoduplex and heteroduplex substrates was about sixfold in the experiment in Table 1; in all experiments it varied between three- and sevenfold. Nevertheless, significant inactivation of native and homoduplex was seen, which could be due to contaminating nuclease activity. This possibility, however, seems very unlikely on the basis of results from experiments done to dissociate the nonspecific activity (on homoduplex or native DNA) from the specific activity (on heteroduplex DNA). In experiments in which the salt concentration of the reaction mixture was varied, or different dilutions of the enzyme were used, reduction or loss in specific activity led to a simultaneous reduction or loss of the nonspecific activity. It therefore seems probable that a proportion of the native and homoduplex molecules are imperfect structures. The process of hybridisation may increase the proportion of defective molecules and therefore tend to minimise the difference between homoduplex and heteroduplex

Table 1 Inactivation of native, homoduplex and heteroduplex DNA by DNase I

Type of DNA	Infective centres		% Surviving Biological activity
	No enzyme	+ enzyme	
Native SPPI	2.4×10^5	1.2×10^5	47.6
161/161 } homoduplex	1.6×10^4	4.0×10^3	24.5
+/+ } homoduplex	2.7×10^4	7.2×10^3	26.6
161/+ } heteroduplex	3.3×10^4	1.2×10^3	3.6
+ /161 } heteroduplex	2.1×10^4	8.2×10^3	3.9

The *Ustilago* enzyme was purified according to the procedure of Holloman and Holliday¹⁵, and fraction V was used in all experiments. A solution containing 7,000 U ml⁻¹ was diluted 1/10 in 10 mM Tris (pH 7.5) and 5 μ l was incubated with 0.1 ml DNA (5 μ g ml⁻¹; saturating conditions in transfection) and 0.1 ml of 10 mM MgCl₂ for 15 min at 37 °C. The reaction was terminated by adding 0.2 ml competent cells (a dilution effect). After addition of competent cells the standard transfection procedure was followed¹⁹. Each number represents infective molecules per ml, individual values being obtained from a total plaque count of ~600-800.

*Present address: Department of Internal Medicine, School of Medicine, Yale University, 333 Cedar Street, New Haven, Connecticut 06520.

Table 2 The effect of DNase I on deletion heteroduplex and homoduplex DNA

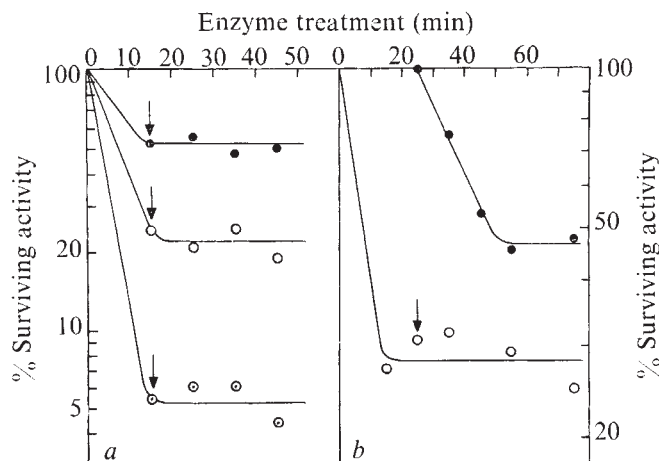
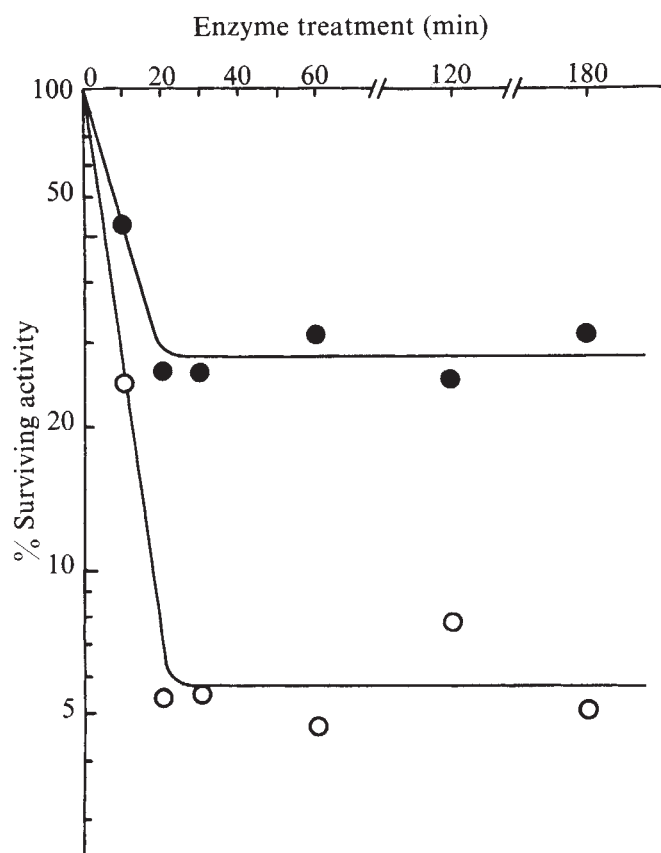
Type of DNA	Infective centres		% Surviving Biological activity
	No enzyme	+ enzyme	
4307/4307	2.5×10^3	1.7×10^3	70.3
+ /4307 } deletion	1.8×10^4	2.1×10^3	11.3
4307/+ } hets	6.5×10^3	1.2×10^3	18.9
+ /161 } point mutant	4.6×10^4	2.2×10^4	48.0
161/+ } hets	3.2×10^3	1.7×10^3	53.6

All types of DNA incubated for 15 min at 37 °C with 1/20 dilution of enzyme solution.

inactivation. Heteroduplexes containing the deletion 4307 are inactivated extremely rapidly in comparison with those containing a single base-pair mismatch (Table 2), suggesting a correlation between the distortion in the DNA and enzyme inactivation.

The kinetics of nuclease activity on homoduplex and heteroduplex DNA were followed by mixing DNA and enzyme at zero time. At appropriate intervals thereafter, samples were removed, transferred to tubes containing competent cells and incubated for 30 min at 37 °C to allow for maximum DNA uptake. The results of one such experiment are given in Fig. 1. The initial rate of heteroduplex inactivation was more rapid than with the homoduplex, but both types of DNA reached maximum, but different, inactivation levels after 15–20 min with DNase I. A proportion of the DNA molecules, yielding about 30 % infective centres (homoduplex) and about 5 % infective centres (heteroduplex), seemed to become resistant to the nuclease (see also Table 1).

The plateau in Fig. 1 could be due to a loss of enzyme activity after 15 min incubation or to a fraction of DNA molecules which is resistant to inactivation by the enzyme. The first

Fig. 1 Kinetics of enzyme inactivation of homoduplex (●) and heteroduplex (○) DNA.**Fig. 2** *a*, Inactivation of native (●), homoduplex (○) and heteroduplex (○) DNA. The arrows indicate time of addition of further enzyme (5 μ l 1/10 dilution). *b*, Initial inactivation of homoduplex (○) DNA, followed by inactivation of native DNA (●) added after 25 min.

alternative was excluded by adding fresh enzyme to three types of DNA that had already been incubated with the enzyme for 15 min. No further decrease in biological activity was observed (Fig. 2*a*). The second alternative was supported by the following experiment. We first incubated homoduplex DNA (161/161) and *Ustilago* DNase for 25 min, then added wild-type native SPP1 DNA and subsequently followed the kinetics of inactivation of both 161 DNA and wild type DNA. In SPP1 the wild type plaques, which form a halo, are easily distinguishable from the 161 plaques, which are haloless. This enabled us to follow the kinetics of inactivation of both mutant and wild type DNA simultaneously. Figure 2*b* shows that active nuclease molecules were present in the enzyme–DNA mixture that could not degrade the initial substrate any further, but efficiently attacked any new substrate that was presented.

As we have mentioned, DNase I requires free ends of DNA for activity¹⁶. It is possible that after binding to an end the enzyme diffuses along the DNA, introducing nicks at points where normal Watson–Crick base pairing is disturbed. We suggest that the preparation of DNase I we used contained a small fraction of defective or partially denatured molecules which bind to the ends of double-stranded DNA, but remain attached there. These would then prevent normal enzyme gaining access to the internal regions of the DNA. If our interpretation is correct, then the difference in the resistant fraction of native (~50 %) and heteroduplex DNA (~5 %) implies that normal enzyme molecules often monitor the former without introducing a nick. Alternatively, a population of DNA molecules which all contain a single-strand nick could have a residual biological activity of ~5 %. We do not yet have enough data to distinguish between the two possibilities.

We have previously compared the relative sensitivity of irradiated and unirradiated native T7 DNA to the *Ustilago* nuclease. Alkaline sucrose sedimentation patterns show that the enzyme prefers a substrate containing dimers¹⁴. In the SPP1 transfection system one can irradiate either heavy or light strands before hybridisation (the H strand has 20 % more pyrimidines than the L strand). As Fig. 3 shows, such hybrids when incubated with DNase I show a greater loss of biological activity than the untreated hybrids. In addition, we see a greater degree of inactivation when the irradiated strand is heavy.

Specificity and biological role of the enzyme

It has been shown that a nuclease isolated from *Neurospora* recognises and degrades single-stranded regions in deletion–wild type heteroduplexes²¹. Recently a similar nuclease (S1), isolated from *Aspergillus*¹⁸, was also shown to be specific for

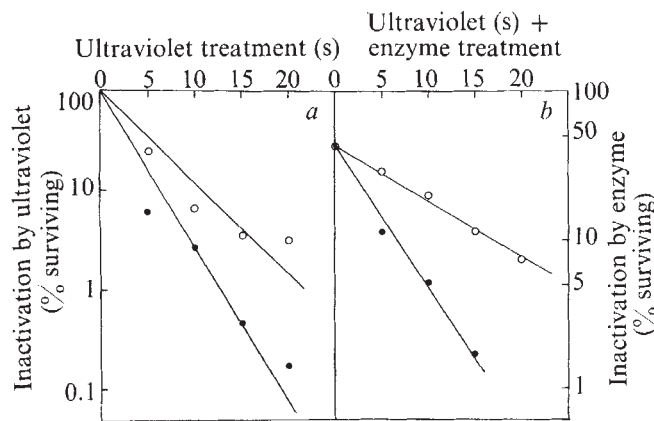


Fig. 3 Inactivation of DNA by ultraviolet light followed by DNase treatment. Single strands, either heavy (●) or light (○), were exposed to ultraviolet light (Sterisol, Hanau lamp, emitting $1.1 \text{ J m}^{-2} \text{ s}^{-1}$ at 30 cm). After hybridisation, DNA was treated for 15 min with DNase (as described in Table 1). *a*, Inactivation by ultraviolet without enzyme treatment; *b*, additional inactivation of the same substrates by a constant enzyme treatment.

mismatch-containing substrates²². The involvement of these enzymes in genetic processes is unknown because of the lack of mutants deficient in the enzymes. In the case of *U. maydis*, however, there is clear evidence that mutant strains which have a reduced amount of the enzyme in crude extracts have a barely detectable level of gene conversion^{13,14}.

The results we have obtained demonstrate that DNA containing a single purine-pyrimidine mismatch, which presumably produces a very minor distortion in DNA, is a preferred substrate to an identical one without the mismatch. A deletion-wild type heteroduplex is even more sensitive to the enzyme, possibly due to a greater distortion in the DNA. It is perhaps significant that addition or deletion mutants of *Ascobolus immersus* show gene conversion much more frequently than do base substitution mutants¹². With the latter, postmeiotic segregation often occurs, suggesting that the correction enzyme is not very efficient at recognising these minor distortions in hybrid DNA.

The discovery of an enzyme which can recognise DNA containing a single mismatch provides strong evidence for the hypothesis that gene conversion is due to the correction of mismatched bases in heteroduplex DNA *in vivo*. As in ultraviolet repair by excision of pyrimidine dimers, it is likely that the overall correction process involves a mismatch-provoked degradation and resynthesis of a short region of single-stranded DNA^{1,23,24}. DNase I may be involved only in the first step of this process. In the absence of information concerning the chemical nature of the degradation products of the enzyme, we cannot

say anything about the actual event which leads to biological inactivation in our system.

The knowledge that enzymes exist which are specifically involved in mismatch repair leads one to consider the biological function or significance of such a correction mechanism. It is believed that incorrect bases may be removed at the replication fork by the editing function of DNA polymerases²⁵. If this editing fails, however, and an incorrect base is inserted into DNA, correction enzymes such as DNase I could come into operation. Random repair provides no biological advantage over the segregation of mutant and wild-type strands after replication. One would therefore need to assume non-random repair leading to the excision of the newly synthesised 'wrong' strand. This could conceivably happen at the replication fork, where discontinuous synthesis would provide the necessary ends required for nuclease activity. If these nucleases are involved in general repair, it would be expected that mutants lacking such repair would have a mutator phenotype. Evidence suggests that *Pneumococcus* strains lacking the capability for heteroduplex repair have an abnormally high rate of spontaneous mutation²⁶. An alternative or additional function of a correction enzyme could be the recognition and removal of damaged or abnormal bases in DNA, such as uracil, derived from the spontaneous deamination of cytosine²⁷. Enzymes which recognise base pairs such as uracil-guanine may also detect other abnormal base-pair combinations.

We thank Miss Brigitte Diering for technical assistance. We also thank Thomas A. Trautner for useful criticisms and suggestions in preparing the manuscript.

Received July 28; accepted September 12, 1975.

- ¹ Holliday, R., *Genetics*, **78**, 273-287 (1974).
- ² Broker, T. R., and Lehman, I. R., *J. molec. Biol.*, **60**, 131-149 (1971).
- ³ Meselson, M. S., and Radding, C. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 358-361 (1975).
- ⁴ Whitehouse, H. L. K., *Towards an Understanding of the Mechanism of Heredity*, 2nd ed. (Arnold, London, 1969).
- ⁵ Kushev, V. V., *Mechanisms of Genetic Recombination* (Plenum, New York, 1974).
- ⁶ Holliday, R., *Genet. Res.*, **5**, 282-304 (1964).
- ⁷ Whitehouse, H. L. K., *Nature*, **199**, 1034-1040 (1963).
- ⁸ Spatz, H. C., and Trautner, T. A., *Molec. gen. Genet.*, **109**, 84-106 (1970).
- ⁹ Wildenberg, J., and Meselson, M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2202-2206 (1975).
- ¹⁰ Nevers, P., and Spatz, H. C., *Molec. gen. Genet.*, **139**, 233-243 (1975).
- ¹¹ Roger, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 466-470 (1972).
- ¹² Leblon, G., *Molec. gen. Genet.*, **115**, 36-38; **116**, 322-335 (1972).
- ¹³ Badman, R., *Genet. Res.*, **20**, 213-229 (1972).
- ¹⁴ Holliday, R., Holloman, W. K., Banks, G. R., Unrau, P., and Pugh, J. E., in *Mechanisms in Recombination* (edit. by Grell, R. F.), 239-262 (Plenum, New York, 1974).
- ¹⁵ Holloman, W. K., and Holliday, R., *J. biol. Chem.*, **248**, 8107-8113 (1973).
- ¹⁶ Holloman, W. K., *J. biol. Chem.*, **248**, 8114-8119 (1973).
- ¹⁷ Linn, S., and Lehman, I. R., *J. biol. Chem.*, **240**, 1294-1304 (1965).
- ¹⁸ Ando, T., *Biochim. biophys. Acta*, **114**, 158-168 (1966).
- ¹⁹ Trautner, T. A., and Spatz, H. C., *Curr. Top. Microbiol. Immun.*, **62**, 61-88 (1973).
- ²⁰ Trautner, T. A., Spatz, H. C., Behrens, B., Paevele, B., and Behncke, M., *Adv. Biosci.*, **8**, 79-87 (1972).
- ²¹ Bartok, K., Garon, C. F., Berry, K., Fraser, M. J., and Rose, J. A., *J. molec. Biol.*, **87**, 437-449 (1974).
- ²² Shenk, T. E., Rhodes, C., Rigby, P. W. J., and Berg, P., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 989-993 (1975).
- ²³ Fincham, J. R. S., and Holliday, R., *Molec. gen. Genet.*, **109**, 309-322 (1970).
- ²⁴ Leblon, G., and Rossignol, J. L., *Molec. gen. Genet.*, **122**, 165-182 (1973).
- ²⁵ Brutlag, D., and Kornberg, A., *J. biol. Chem.*, **247**, 241-248 (1972).
- ²⁶ Tiraby, J. G., and Fox, M. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3541-3545 (1973).
- ²⁷ Lindahl, T., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3649-3653 (1974).

letters to nature

New non-stellar BL Lacertae objects OY091 and PKS2335+03

DURING the past year, opinions on the nature of the BL Lacertae objects have narrowed to the association of elliptical galaxies with non-thermal sources which display continuous optical spectra. The study of the objects B2 1101+38 (Markarian 421) and B2 1652+39 (Markarian 501)

indicates that they could be members of a sequence of elliptical galaxies ordered by the relative brightness of the BL Lac objects located in their nuclei¹. On the other hand, the proximity of B2 1215+30, ON231, PKS1205-008 and PKS0048-097 to galaxies or nebulosities, has prompted the suggestion that, at least these four BL Lac objects have been ejected from the nuclei of their companion galaxies (J. J. Condon and D. L. Jauncey, unpublished). Furthermore, an analysis of the optical energy distribution² of

Table 1 Details of exposure

Exposure (min)	Filter	Emulsion
8	RG-2	103a-D
15	6,000-7,200Å (HBW)	103a-D
30	RG-2	IIIa-J

several BL Lac objects indicates that the decline of their optical spectra toward the ultraviolet becomes steeper because of the contribution of underlying elliptical galaxies. Typical examples are Markarian 501, AP Librae and BL Lac itself. In the case of AP Librae, the nebulosity was discovered with deep image tube photographs¹.

We undertook a search for nebulosities underlying the BL Lac objects 4C14.60, OT081, OY091 and PKS2335+03 and obtained positive results in the last two cases. These four objects appear stellar on glass copies of the National Geographic-Palomar Observatory Sky Survey. The selection of these objects was based on the steep decline of their spectra towards the ultraviolet². Our observations were made with a 140-mm ITT image tube camera with a red-extended S-20 spectral response. The camera was used at the Cassegrain focus of the Steward Observatory 2.25-m telescope to obtain a 23' field with a 10" mm⁻¹ scale. On the nights of August 2 and 3, 1975, we made three exposures of each of these four objects (see Table 1).

The objects 4C14.60 and OT081 remain stellar in appearance to the limit of our plates ($V \sim 22.5$ mag); however, both OY091 and PKS2335+03 exhibit non-stellar images on all of our plates. OY091, shown in Fig. 1, has a very soft image, with surrounding nebulosity highly reminiscent of that seen on deep plates of AP Librae³. Transmission scans in one dimension across the disk of OY091 clearly confirm the non-stellar image. The object PKS2335+03, shown in Fig. 2, is perhaps more interesting because the observed structure is clearly extended to the east, although there is also some fainter structure to the west. The distinct eastward extension resembles a jet or an extremely close companion.

The fields around OY091, PKS2335+03 and 4C14.60 contain many faint galaxies, suggesting a possible membership

Fig. 1 This reproduction of the field of OY091 is from a 30-min exposure with an RG-2 filter on a IIIa-J emulsion. North is at the top; east is to the left.

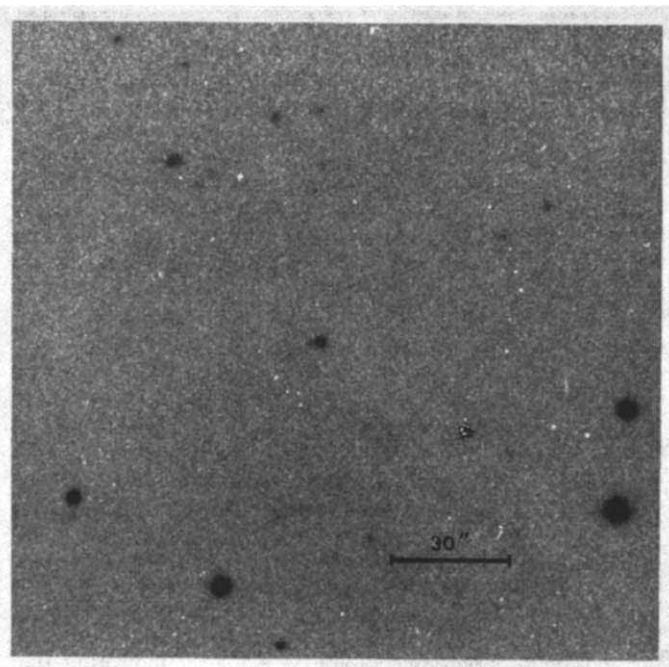


Fig. 2 PKS2335+03 is shown with the nebulous extension to the east in this reproduction of an 8-min exposure with an RG-2 filter on a 103a-D emulsion. Orientation as in Fig. 1.

of these three objects with distant clusters of galaxies. In this respect OY091, PKS2335+03 and 4C14.60 are similar to PKS0548-322, which has been found to be projected on to a cluster of galaxies⁴. No evidence for a cluster of galaxies is observed on our plates of OT081; however, the low galactic latitude ($b^{\text{II}} = 17.7^\circ$) of this object, precludes a definitive statement. The association of BL Lac objects with clusters of galaxies is a matter which deserves further study.

The morphological classification of BL Lac objects suggests three possible groups: (1) objects located in galaxies, (2) objects associated with nearby galaxies and (3) isolated objects. Since the majority of the BL Lac objects seem to be somehow related to galaxies, it seems of interest to ask whether isolated BL Lac objects really exist, or will they eventually be found to be associated with galaxies? Because the ability to detect faint nebulosity, using photographic techniques, is a strong function of the distance to the object, it would be useful to find other observational parameters to detect associated galaxies. When a BL Lac object is located in a galaxy, the steepness of the optical energy distribution seems to be a useful parameter. The detection of underlying nebulosity in OY091 and PKS2335+03, two objects with spectral indices ($d \log F_\nu / d \log \nu$) of -2.7 and -2.3 respectively², tends to confirm this suggestion.

We thank R. Schiff of Steward Observatory for preparation of the photographic material. M. T. acknowledges support from an ESRO fellowship.

ERIC R. CRAINE
S. TAPIA
M. TARENGHI

Steward Observatory,
University of Arizona,
Tucson, Arizona 85721

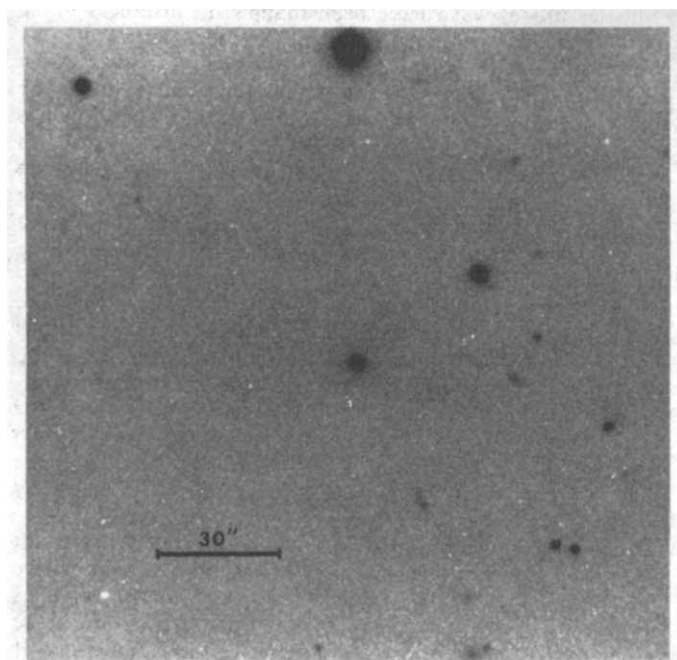
Received August 11; accepted September 30, 1975.

¹ Ulrich, M.-H., Kinman, T. D., Lynds, C. R., Rieke, G. H., and Ekers, R. D., *Astrophys. J.*, **198**, 261-266 (1975).

² Tapia, S., Craine, E. R., and Johnson, K., *Astrophys. J.*, (in the press).

³ Disney, M. J., Peterson, B. A., and Rodgers, A. W., *Astrophys. J. Lett.*, **194**, L79-L82 (1974).

⁴ Disney, M. J., *Astrophys. J. Lett.*, **193**, L103-L105 (1974).



Cosmic rays and interstellar tunnels

MEASUREMENTS have been made¹ of the isotopic abundances of Be in cosmic rays, which have led to the conclusion that the typical cosmic ray lifetime in the galaxy is $\sim 2 \times 10^7$ yr. Furthermore, measurements² of the cosmic ray electron energy spectrum in the range 6–100 GeV, have shown a bend in the spectrum which can be attributed to inverse Compton and synchrotron losses. The position of the bend is indicative of the cosmic ray electron lifetime, and an age of 1.2×10^7 yr is derived.

These two values are significantly longer than the widely quoted value of $\sim 3 \times 10^6$ yr. This latter value is consistent with the average column density of material traversed by cosmic rays, $\sim 5 \text{ g cm}^{-2}$ (as determined by measurements of products of spallation reactions), and the usually assumed average density of matter in the galactic disk ($\sim 1 \text{ H atom cm}^{-3}$).

Measurements of the interstellar density indeed show³ $\rho_{\text{is}} \sim 1$. The newly determined cosmic ray lifetime, however, implies that the average density through which the particles have travelled is $\sim 0.2 \text{ atom cm}^{-3}$. This value is an upper limit to the average density encountered by cosmic rays in the galaxy at large, since there is evidence indicating that cosmic rays traverse a large fraction of the above mentioned column density at the particle sources⁴. It is therefore apparent that cosmic rays must spend $\sim 80\%$ of their lifetimes in regions of the galaxy where the density is significantly less than the average value found in the disk.

For this reason, the existence of a cosmic ray "halo" has been postulated^{5,6}. This halo is a low-matter density region of cosmic ray confinement which surrounds the galactic disk.

Webster⁶, however, analysed data concerning the radio halo of the galaxy, and found that the idea of static confinement in the halo is inconsistent with the observations. Dynamic confinement such as that proposed by Jokipii⁵ may explain the low mean density traversed by cosmic rays.

An alternative explanation is proposed here. The low mean density traversed may be indicative of the presence of hot, low density tunnels in the interstellar medium such as those proposed by Cox and Smith⁷. Cosmic rays produced in supernovae or in supernova remnants are preferentially injected into these tunnels. The tunnels act as cosmic ray traps in which the density is low ($\sim 10^{-3} \text{ atom cm}^{-3}$), thus reducing the mean density encountered over the particle lifetime.

Many of the theories of the origin of cosmic rays involve particle acceleration by supernovae explosions, by the resultant pulsar, or in the supernova remnants. Analysis of cosmic ray abundances⁸ supports the view that cosmic rays are accelerated, at least partially, in supernova remnants (compare with ref. 9 and J. R. Jokipii, personal communication). Indeed, γ -ray observations of the Vela supernova remnant¹⁰ show that it is likely that supernovae or supernova remnants both produce and confine enough cosmic rays to explain the observed galactic cosmic ray density.

Cox and Smith⁷ estimate that a fraction, f , of the volume of the interstellar medium is occupied by hot, tenuous tunnels caused by linked chains of supernova remnants, where $0.1 \lesssim f \lesssim 0.5$. Since typical remnants expand to a radius of $\sim 40 \text{ pc}$ before they start to disappear, they have a probability, P , of intersection with an existing tunnel, such that $0.6 \lesssim P \lesssim 1$. When the shock front of a new remnant encounters an existing tunnel, the shock is preferentially driven along the tunnel. Thus the remnant material which contains the cosmic rays produced by the supernova (Higdon and Lingenfelter's¹⁰ data indicate that cosmic rays are confined up to a remnant radius of $\lesssim 30 \text{ pc}$) is injected into the tunnel system.

As was pointed out previously⁷, the tunnel systems are excellent cosmic ray traps, since they are regions of low magnetic field. The tunnels are intertwined about the large scale flux tubes of the galactic magnetic field. Particles injected into the system

are therefore internally reflected by the high magnetic fields in the tunnel walls. The particles can escape only by slowly diffusing on to the galactic field lines.

The most likely mechanism of particle escape from the tunnel is resonant pitch-angle scattering with turbulent fluctuations in the interstellar field. The typical ($\sim 3 \text{ GeV}$) cosmic ray particle has a gyro radius $\sim 10^{12}$ – 10^{13} cm in the galactic field ($\sim 3 \times 10^{-6} \text{ gauss}$). Unfortunately, we have no observations of the scale size of the turbulence in this regime, and this makes it difficult to estimate the time scale of escape. It is, however, interesting to note that this mechanism will, if operative, give an energy dependence to the cosmic ray spallation products. This is because particles with different gyro radii (that is, different energies) will scatter most effectively off correspondingly different sized fluctuations, and the probability of encountering a given size fluctuation is generally a function of the size of that particular turbulent element. This effect produces a variation in the fraction of the lifetime of the particles which is spent in the high density regions of the disk, and thus a variation in the spallation products. Energy dependence of spallation products is indeed observed¹¹, although an alternative explanation has been suggested⁹.

JOHN S. SCOTT

Kitt Peak National Observatory,
Tucson, Arizona 85726

Received August 20; accepted September 11, 1975.

- ¹ Garcia-Munoz, M., Mason, G. M., and Simpson, J. A. (in the press).
- ² Meegan, C. A., and Earl, J. A. (in the press).
- ³ Jenkins, E. B., and Savage, B. D., *Astrophys. J.*, **187**, 243–256 (1974).
- ⁴ Arnett, W. D., and Schramm, D. N., *Astrophys. J. Lett.*, **184**, L47–52 (1973).
- ⁵ Jokipii, J. R. (in the press).
- ⁶ Webster, A., *Mon. Not. R. astr. Soc.*, **171**, 243–257 (1975).
- ⁷ Cox, D. P., and Smith, B. W., *Astrophys. J. Lett.*, **189**, L105–108 (1974).
- ⁸ Haebich, K. L., Norman, E. B., and Schramm, D. N., *Astrophys. J.* (in the press).
- ⁹ Scott, J. S., and Chevalier, R. A., *Astrophys. J. Lett.*, **197**, L5–8 (1975).
- ¹⁰ Higdon, J. C., and Lingenfelter, R. E., *Astrophys. J. Lett.*, **198**, L17–20 (1975).
- ¹¹ Cesarsky, C. J., and Audouze, J., in *Proc. 13th Int. Cosmic Ray Conf.*, 1 (Denver, 1973).

Measurements of the far-field resonance cone for whistler mode waves in a magnetoplasma

USING a large volume laboratory magnetoplasma the wave fields and resonance cone of the right hand polarised (whistler mode) wave have been mapped at distances of up to 10 whistler wavelengths from a transmitting antenna. The results are in good agreement with theory and suggest that energy is convected away from the antenna along the surface of the resonance cone. Considerable attention has been paid to the generation of waves by a point source in a magnetoplasma, and theoretical analyses^{1–5} have shown that for certain combinations of the antenna operating frequency ω , the electron gyrofrequency ω_c , and the plasma frequency ω_p , the fields become singular along a cone whose axis is parallel to B_0 , the steady magnetic field. By examining the refractive index surfaces it can be seen that the group velocity of the wave is zero when the ray vector is parallel to the resonance cone of half angle θ (when the wave normal is at an angle $\pi/2 - \theta$ to B_0). We restrict ourselves here to a discussion of the radiation pattern for frequencies $\omega < \omega_c \ll \omega_p$. Waves launched by the antenna then propagate in the right hand polarised whistler mode and the angle of the resonance cone is approximately given by $\theta = \sin^{-1}(\omega/\omega_c)$.

This phenomenon is of particular interest because it defines a narrow region of space in a magnetoplasma where the group velocity is very small. The question then arises as to whether energy is, in fact, transferred along the resonance cone or is merely a near-field phenomenon. If

the cone exists in the far field it would have a significant effect on the propagation characteristics of the wave and the impedance of the transmitting antenna. This information would be needed with any mother-daughter spacecraft experiment where the dispersion of a wave travelling between two spacecraft is interpreted in terms of the local plasma parameters. It is also needed in laboratory experiments on controlled thermonuclear reactions where radio frequency energy is used to heat the charged particles to high temperatures.

The behaviour of the cone angle as a function of ω , ω_c and ω_p has been studied experimentally in the near field^{5,6} and is reasonably well understood. The far field of the cone and wave fields have been studied theoretically⁷ but little

The experiment was performed in a large volume magnetoplasma⁸, 60 cm in diameter and approximately 150 cm long. An axial magnetic field was provided by three solenoids giving an axial uniformity of $\pm 0.6\%$ over 120 cm and 0.01% uniformity over the central 10 cm. Fields of up to 300 gauss can be obtained (see Fig. 1).

A plasma was formed in a glass tube 15 cm in diameter at one end of the vacuum chamber by radio frequency ionisation at 80 MHz. The oscillator produced about 1 kW of radio frequency power which was coupled into the plasma inductively. The coupling between the source axial magnetic field and the main field allowed the plasma to drift into the experimental volume, producing a radial gradient in density, which was approximately Gaussian.

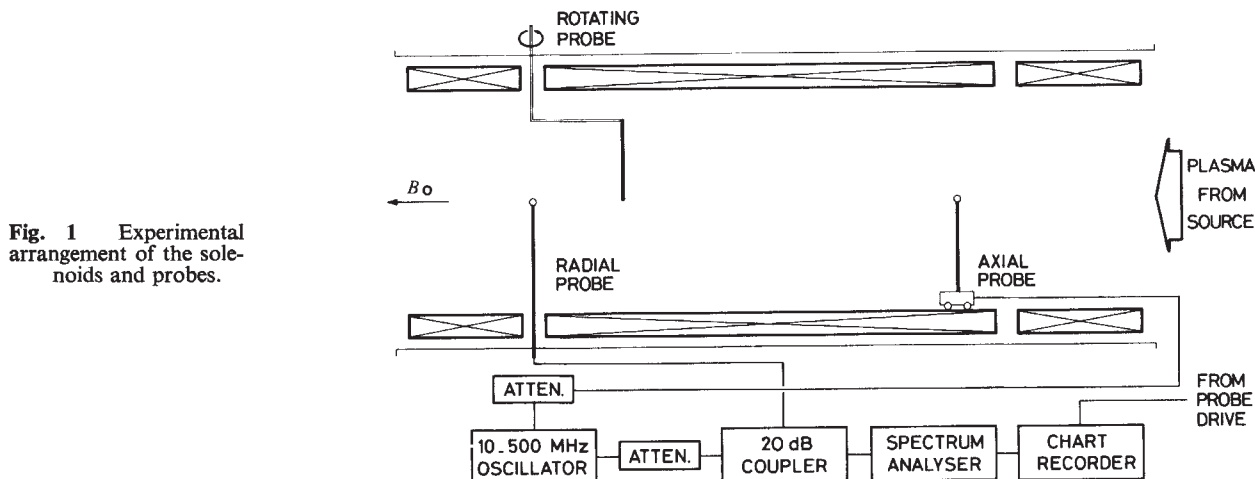


Fig. 1 Experimental arrangement of the solenoids and probes.

other work has been done on this part of the problem. In particular, the shape of the wave fronts in the cone and the propagation of energy along the cone surface are problems yet to be solved.

Using small, movable probes in a large, laboratory magnetoplasma, I mapped the radiation field of an antenna over a distance of up to 10 wavelengths of the whistler wave. For convenience, a frequency of about half that of ω_c was used since it was thought that the focusing effect predicted by Wang and Bell⁷ may be observable, although they investigated the very far-field radiation pattern at a distance of approximately 6,000 wavelengths.

Langmuir probe measurements gave the electron temperature as 2.6 eV, in good agreement with results derived from measurements of the interference structure of the resonance cone⁹. For this experiment the magnetic field was set at 64 gauss, with an Argon gas pressure of 4×10^{-4} mmHg giving a collision frequency—essentially caused by electron—neutral collisions—of about $0.0005 \omega_c$. Any dissipative effects would, therefore, be of a collisionless nature.

By measuring the whistler wavelengths over two decades in frequency a graphical plot of the dispersion was obtained. This was compared with theoretical curves using ω_p as a fitted parameter, and yielded an average value for the electron density of $4 \times 10^{10} \text{ cm}^{-3}$.

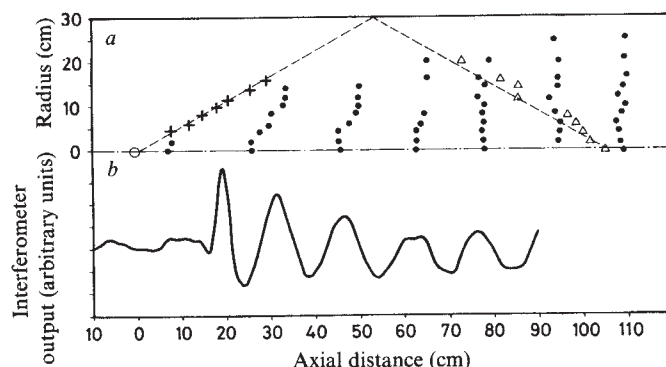
The transmitting antenna had a loop 1 cm in diameter at the end of a coaxial probe 3 mm in diameter which could be moved along the length of the vessel. Receiving antennae were similar loops or small electric probes with an exposed surface 1 mm in diameter and 4 mm long. The wave fields were mapped by setting the receiver at a certain radial position and moving the transmitter axially.

Since it was found that the resonance cone itself was reflected from the metal walls of the vessel, this method of moving the transmitter on the axis maintained symmetry and allowed the position of the reflected cone field to be determined easily.

A simple interferometer was constructed using the received signal as one arm and a direct signal from the oscillator driving the transmitting antenna as the other arm. The two signals were mixed and displayed on a spectrum analyser set to zero scan (that is, operating as a narrow band superheterodyne receiver) and a permanent copy was made on an x-y chart recorder.

Figure 2a shows results taken from interferometer traces using a loop receiving antenna for a frequency of 150 MHz

Fig. 2 a Results derived from the interferometer measurements: crosses, positions of the resonance cone; triangles, positions of the cone after reflection from the wall of the vessel; dots, surfaces of constant phase of the whistler wave (each set is 2π in phase apart). b, An interferometer trace taken at a radial distance of 10 cm.



($0.47 \omega_c$). The positions of the reflected cone, shown by the triangles, could be determined from the traces as a rapid change of phase which could be distinguished easily from the whistler wave fields. For this particular wave frequency the theoretical cone angle is 28° , in good agreement with the experimentally measured value of $28 \pm 1^\circ$.

At higher magnetic fields, B_0 , we have measured the resonance cone field at distances greater than 10 wavelengths from the transmitter, where the wavelength referred to is that for a longitudinally propagating whistler wave. This would seem to suggest that energy does in fact propagate along the cone surface away from the transmitter in contrast to the assumption of Wang and Bell⁷ that the radiation intensity near the cone has little physical significance.

Within the cone, the wavefront can be seen to be curved. By examining the refractive index surface and constructing wavefronts, a theoretical wave front can be determined, and that is in good agreement with our measured results. Further away from the cone, the effects of the cylindrical geometry must be taken into account. As the diameter of the vessel is much larger than the wavelength, the dispersion of the waves is little changed but the shape of the wavefront is planar in cylindrical geometry and the gradual change from curved to planar can be seen in the figure.

A maximum variation of about 20% in wavelength along the axis shows that the axial gradient in density caused by the drifting plasma is probably of the same order of magnitude. The radial gradient in density does not seem to have any significant role in the formation of the wavefronts.

An interferometer trace using a loop receiving antenna taken at a radial distance of 10 cm is shown in Fig. 2b. The first maximum corresponds to the resonance cone and following maxima are caused by the wavefields. The non-zero signal to the left of the resonance cone is caused by the interferometer, the wave field outside the cone being essentially zero. By moving a probe radially, the wave fields were found to be greatest along the axis, decreasing gradually as the resonance cone is approached. Although we cannot regard this as a measurement of the focusing effect suggested by Wang and Bell⁷, it is quite possible that after many more wavelengths the fields would be concentrated about the axis.

The increasing area of the wavefront as it moves away from the transmitter can be seen as a gradual decrease in the wave amplitude.

I am at present mapping the radiation fields and, in particular, the relative amplitudes phases and directions of E and H for a wide range of frequencies below the electron gyrofrequency, especially below $\omega = 0.2\omega_c$ where the ray direction can theoretically be outside the resonance cone. These results will be presented elsewhere.

Discussions with A. Gonfalone, D. Jones and A. Pedersen, and the technical assistance of H. Arends, are acknowledged. I also thank Professor T. Kaiser for useful suggestions and comments. I was an ESRO research fellow.

R. W. BOSWELL

SPP-SSD, ESTEC,
Domeinweg,
Noordwijk aan Zee,
The Netherlands

Received June 11; accepted September 23, 1975.

- ¹ Kaiser, T. R., *Planet. Space Sci.*, **9**, 639 (1962).
- ² Kaiser, T. F., and Tunaley, J. K. E., *Space Sci. Rev.*, **8**, 32 (1968).
- ³ Kuehl, H. H., *Phys. Fluids*, **17**, 1275 (1974).
- ⁴ Singh, N., and Gould, R. W., *Radio Sci.*, **6**, 1151 (1971).
- ⁵ Fisher, R. K., and Gould, R. W., *Phys. Fluids*, **14**, 857 (1971).
- ⁶ Gonfalone, A., *J. Phys. (Fr.)*, **33**, 521 (1972).
- ⁷ Wang, T. N. C., and Bell, T. F., *J. geophys. Res.*, **77**, 1174 (1974).
- ⁸ Boswell, R. W., and Gonfalone, A., *Phys. Lett.*, **51A**, 485 (1975).

Subducted greywacke in the Olympic Mountains, USA—implications for the origin of Archaean sodic gneisses

IN Archaean greenstone belts the earliest granitic rocks are sodic or quartz dioritic gneisses and migmatites that surround and diapirically intrude the greenstones. The granitic rocks have been considered as the sialic basement on which the greenstones were deposited^{1,2} and as sialic underplatings of a primordial greenstone crust³⁻⁵. But the structural relationships between greenstones and greywackes in the Olympic Mountains of Washington State, USA, suggest that the Archaean gneisses may have formed by subduction of greywacke suites and associated volcanic rocks beneath nearly coeval oceanic crust.

If the Archaean granitic rocks are primary sialic basement^{1,2} the sodic gneisses must be older than, and beneath, the oldest Archaean greenstones. This possibility is attractive, since gneiss domes in younger terrains are generally regarded as remobilised basement rocks, and the sodic migmatites are compositionally similar to the diapiric plutons⁴. Contacts between the oldest greenstones and sodic granitic rocks are, however, invariably tectonic or intrusive. No unequivocal examples are known where the oldest greenstones lie unconformably over granitic basement⁴⁻⁷. Unconformities do exist within the Archaean, but the basal layers comprise sedimentary rocks, mostly conglomerates, resting on granitic rocks⁶⁻⁸. Further, 'granitic' clasts in Precambrian volcanic-sedimentary belts do not occur in the basal greenstones, and clasts higher up in the sequences may have been derived from nearly contemporaneous volcanic and hypabyssal rocks rather than from a pre-existing granitic basement⁹.

The Archaean gneisses are, admittedly, of higher metamorphic grade and more highly deformed than the adjacent greenstone belts, but these criteria cannot be used reliably to establish the relative ages of suites that are tectonically juxtaposed. Within the Barberton greenstone belt of South Africa, the Swaziland Supergroup has been dated⁴, using Rb/Sr techniques at 3,355 Myr and the surrounding Ancient Gneiss Complex⁴ at 3,395–3,138 Myr. This coeval relationship holds for several Archaean greenstone and gneiss terrains⁵.

The sodic granitic rocks have also been considered to be sialic underplating (without any supracrustal manifestations) of the primordial greenstone crust^{2,4,5}. The granitic migmatitic terrains, however, contain relict metasedimentary or metavolcanic features, such as banded iron formations, amphibolites, and quartzites^{1,4-6}. If the tonalitic bodies within the migmatitic complex adjacent to the Barberton Greenstone Belt were anatectic additions to the gneisses, the resultant increase in volume should have deformed the neighbouring greenstone belt⁴. Moreover, the anatectic mechanisms necessary to generate vast amounts of sodic granitic material from oceanic crust or the mantle are somewhat difficult to envisage^{1,3}.

The Olympic Peninsula comprises two almost coeval lithological assemblages (Fig. 1). The mountain core is composed of Eocene–Miocene slate and greywacke with minor lenses of basaltic rocks. These core rocks are strongly folded and faulted and are metamorphosed in the prehnite–pumpellyite to lower greenschist facies¹⁰.

Flanking the mountain core on the north, east, and south is a discontinuous belt of early (?) and middle Eocene marine basaltic rocks, which are succeeded by an Upper Eocene to Miocene marine sedimentary sequence. Although folded and faulted, this succession is generally stratigraphically continuous, and only modestly deformed compared with the core rocks. The basaltic rocks of the

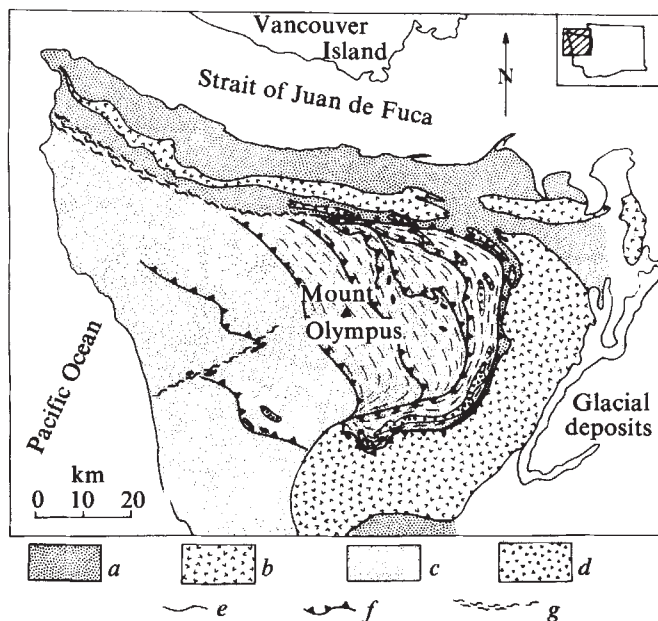


Fig. 1 Structural sketch map of the Olympic Peninsula, Washington (after Tabor¹⁰). *a, b*, Peripheral rocks (orderly, moderately deformed and partially metamorphosed): *a*, Eocene to Miocene sedimentary rocks; *b*, early (?) and middle Eocene volcanic rocks (basalt and greenstone). *c, d*, Core rocks (intensely deformed, faulted, and partially metamorphosed): *c*, Eocene to Miocene sedimentary rocks (dashed where penetratively deformed); *d*, Eocene and younger (?) volcanic rocks (basalts and greenstones). *e*, Contact; *f*, thrust fault; *g*, shear zone.

peripheral belt are dominantly pillowed and massive diabasic oceanic tholeiites^{11,12}, but about 20% are alkalic basalt¹². An intrusive complex of diabase, gabbro, and porphyritic basalt occurs in the thickest portion of the basalts¹².

Chemically, most of the basalts correlate well with oceanic-ridge tholeiites, particularly in their TiO-FeO/MgO distribution and in rare earth elements, although some of the upper flows show trends typical of oceanic-island tholeiites¹². Most of the volcanic pile is apparently a slice of upper oceanic crust, with the small volume of alkalic rocks representing the last phases of volcanism¹².

The volcanic rocks have been statically metamorphosed from zeolite facies to prehnite-pumpellyite and, locally, greenschist facies. Many of the flows "might well be called greenstones"¹³.

The basalts crop out in an arcuate pattern starting on the north-western tip of the peninsula and extending more than 220 km east and south. Their thickness varies from a few tens of metres in the north-west to a maximum of 15 km on the east¹¹. Regionally, these rocks are part of an early (?) to middle Eocene assemblage of marine basaltic rocks that extends from Vancouver Island southwards to the Klamath Mountains, a distance exceeding 600 km.

Structural and palaeontological relationships indicate that the greywackes and slates of the core are of the same age or younger than the basalts and that the core rocks were inserted beneath the peripheral basalts along a thrust mappable over a distance exceeding 120 km^{11,14}. Regional gravity data¹⁵ from the eastern portion of the Olympic Peninsula suggest that the basalts are a slab about 15 km thick, dipping 45 degrees eastwards.

The extreme deformation and thrusting of the core rocks under the peripheral basaltic rocks was probably the result of pre-Pliocene subduction. Subduction is indicated by the ages and patterns of linear magnetic anomalies in the adjacent north-eastern Pacific Ocean¹⁶.

The few published analyses of K₂O-Na₂O ratios in Olympic core rocks^{17,18} and the K₂O/Na₂O plots of Tertiary greywackes, Archaean greywackes, and Archaean sodic granitic gneisses⁵ yield results of about 0.5. Thus, isochemically metamorphosed Tertiary or Archaean greywackes could yield rocks with the same K₂O-Na₂O ratio as Archaean sodic granitic rocks. This suggestion is an extension of the inference¹⁹ that greywackes may be transformed isochemically in to banded albite schists and gneisses. Mineralogically, chemically, and in some cases even texturally, the sodic migmatites are very similar to the sodic diapires that intrude the Barberton Greenstone belt⁴. The presence of basaltic rocks within the greywackes of the core of the Olympic Mountains and of amphibolites in the Precambrian migmatitic terrains is especially interesting in view of Glikson's comment that "Tonalites and granodiorites intruded into Archaean systems commonly abound in basic xenoliths . . .; such altered xenoliths represent remnants of basic rocks intruded by the acid melts thus pointing to an origin of the melts from below a basic crust."³

We concur with Anhaeusser²⁰ that plate tectonic models for Archaean time are viable, and suggest that the Archaean sodic granitic rocks may have been supracrustal rocks that were subducted beneath coeval oceanic greenstones. This hypothesis avoids the problems inherent in postulating a primordial sialic crust, the requirement of large scale anatexitic granitic underplating of the greenstones, and the extreme lateral variations in geothermal gradients otherwise needed⁵ to account for adjacent Archaean belts of vastly different metamorphic grades.

We thank W. R. Dickinson, B. M. Page, C. F. Park Jr, and R. W. Tabor for discussions. This work was supported in part by the National Science Foundation.

ERIC S. CHENEY

RICHARD J. STEWART

Department of Geological Sciences,
University of Washington,
Seattle, Washington 98195

Received April 7; accepted July 22, 1975.

- 1 Anhaeusser, C. R., Mason, R., Viljoen, M. H., and Viljoen, R. P., *Bull. geol. Soc. Am.*, **80**, 2175-2200 (1969).
- 2 Wilson, J. F., *Phil. Trans. R. Soc.*, **A273**, 389-411 (1973).
- 3 Glikson, A. Y., *Bull. geol. Soc. Am.*, **83**, 3323-3344 (1972).
- 4 Hunter, D. R., *Precambrian Res.*, **1**, 259-294 (1974).
- 5 Engle, A. E. J., Itson, S. P., Engle, C. G., Stickney, D. M., and Cray, E. J., *Bull. geol. Soc. Am.*, **85**, 843-858 (1974).
- 6 Windley, B. F., *Phil. Trans. R. Soc.*, **A273**, 321-341 (1973).
- 7 McGlynn, J. C., and Henderson, J. B., *Spec. Pap. geol. Ass. Can.*, **11**, 506-526 (1972).
- 8 Jolliffe, A. W., *Spec. Pap. geol. Ass. Can.*, **3**, 75-98 (1966).
- 9 Ojakangas, R. W., *Bull. geol. Soc. Am.*, **83**, 429-442 (1972).
- 10 Tabor, R. W., *Bull. geol. Soc. Am.*, **83**, 1805-1816 (1972).
- 11 Cady, W. M., Tabor, R. W., MacLeod, N. S., and Sorensen, M. L., *Quadr. Map U.S. geol. Surv.*, **QO 970** (1972).
- 12 Glassley, W., *Bull. geol. Soc. Am.*, **85**, 785-794 (1974).
- 13 Park, C. F., Jr, *Am. J. Sci.*, **244**, 305-323 (1946).
- 14 Tabor, R. W., Yeats, R. S., and Sorensen, M. L., *Quadr. Map U.S. geol. Surv.*, **QO 958** (1972).
- 15 Stuart, D. J., *Prof. Pap. U.S. geol. Surv.*, **424C**, C273-C276 (1961).
- 16 Atwater, T., *Bull. geol. Soc. Am.*, **81**, 3513-3536 (1970).
- 17 Pettijohn, F. J., *Sedimentary Rocks* (Harper and Brothers, New York, 1957).
- 18 Hawkins, J. W., *Am. J. Sci.*, **265**, 798-818 (1967).
- 19 Coombs, D. S., *Mineralog. Mag.*, **34**, 144-158 (1965).
- 20 Anhaeusser, C. R., *Phil. Trans. R. Soc.*, **A273**, 359-388 (1973).

Plate tectonic significance of repeated tectonic trends in eastern Canada

THE existence of generally parallel, major tectonic structures of different ages has been long recognised. In Eurasia, for instance, the Alpine Orogenic Belt is paralleled by the Hercynian-Variscan Orogenic Belt, and in southern New Brunswick there is a general parallelism between the Alleghenian (Variscan) and Acadian fold belts¹. In Newfoundland² the Acadian Fold Belt runs generally parallel to the older, late Cambrian-earliest Ordovician (Grampian) Fold Belt in the west and a pre-middle Ordovician fold belt in the east. Further, the Appalachian-

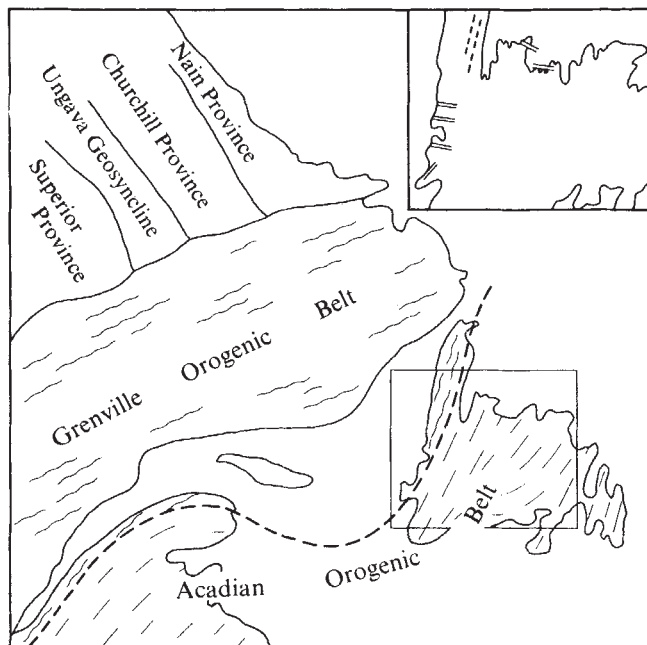


Fig. 1 Tectonic features of eastern Canada. Inset (showing boxed area): dashed lines, dykes cutting the Grenville Province; solid double lines, sheeted dykes.

Caledonian Belt as a whole in north-eastern Canada runs parallel to the older (1,000 Myr BP) Grenvillian Orogenic Belt for at least 1,000 miles. These are clearly indications of continental accretion (Fig. 1).

The Precambrian and Phanerozoic Belts which run parallel to the North American seaboard of the north Atlantic Ocean are terminated transversely by the Labrador Sea. Thus, in terms of pre-Mesozoic fit, the correlation of the Grenville Formation³ with the British Isles is uncertain. The Caledonian and northern Appalachian Orogenic Belts, in spite of their apparent continuity from Newfoundland to the UK, are not of identical ages. The Caledonian Belt generally reached a climactic episode during end-Silurian-early Devonian times, whereas the northern Appalachian Belt was generally deformed during the mid-Devonian. An important factor in the interpretation of the cutoff against the Labrador Sea is the nature of the ophiolite complexes in Newfoundland. Dewey and Bird⁴ have drawn attention to the significance of Newfoundland ophiolites as portions of old (early Ordovician and older) oceanic plate. Later work⁵⁻⁸ resulted in a wider recognition that these ophiolite complexes could be remnants of Ordovician or older small oceans, with an internal structure of oceanic lithosphere, including basaltic pillow lavas, sheeted dykes, gabbros and upper mantle peridotites.

Dewey and Bird's model envisaged that the deformation of the sedimentary prism marginal to the continent was contemporaneous with the subduction of the oceanic crust. In this respect the oceanic crust of the small oceans in Newfoundland needs further study, as it includes some unexplained features. First, the fragments of oceanic crust lie tectonically over⁹ or, at least, almost against the sialic crust¹. Second, their internal structure cannot be related to the deformed sialic rocks since the sheeted dyke complexes are at a high angle to the trend of orogenic deformations (Fig. 1, inset). The direction of the initial spreading of the preserved oceanic crust is thus not parallel to the direction of final closing (Fig. 1, inset) of the ocean as a whole.

The relationship between the ophiolites and the adjacent continental rocks along the northern coast of Newfoundland can be interpreted in terms of obduction, rather than

subduction. The trend of the dykes in the sheeted complexes suggests that the small ocean whose strike is indicated by the sheeted dyke complex, had a trend radically different from the larger ocean to which the Appalachian Orogenic Belt of Newfoundland was marginal. This larger ocean is often referred to as the proto-Atlantic⁹. It is significant that the trend of the Ordovician(?) sheeted dykes is sub-parallel to the present magnetic lineations of the Labrador Sea^{10,11}. It seems reasonable to suggest that the small Ordovician ocean had the same relation to the proto-Atlantic as the present Labrador Sea has to the modern Atlantic Ocean. If that is so, then the preservation of parts of the small ocean (proto-Labrador Sea) could be explained by the hypothesis that, when the proto-Atlantic Ocean closed, a small, oceanic, proto-Labrador Sea became completely land locked. Similarly, were the modern Atlantic Ocean to close, one would expect the oceanic crust of the present Labrador Sea to be preserved at a high tectonic level and to obduct on to the adjacent Greenland-Canadian mainland. It would not subduct because it would be situated inside the subducting margin of the closing Atlantic Ocean.

The hypothesis has further implications, in so far as it suggests that a triple junction of the type that now exists at the meeting point of the North Atlantic Ocean, the north-eastern Atlantic Ocean and the Labrador Sea must have existed at approximately the same location during early Palaeozoic times. Thus, the cutoff of the Grenville Belt becomes more intelligible and the time difference between the Caledonian and Acadian orogenies is explicable by regarding the two branches of the orogenic belt as lying on opposing sides of the triple junction. Furthermore, the Labrador branch of the Ungava Geosyncline¹², deformed during the Hudsonian Orogeny, may have represented a margin to a still earlier 'Labrador Ocean' which existed there some 2,000 Myr BP. It is fitting to suggest a longevity of certain tectonic situations in geological time and the existence of lithospheric weaknesses which are repeatedly subject to separation and collision.

N. RAST

D. E. RAST

Department of Geology,
University of New Brunswick,
Canada

M. J. KENNEDY

Department of Geology,
Memorial University of Newfoundland,
Canada

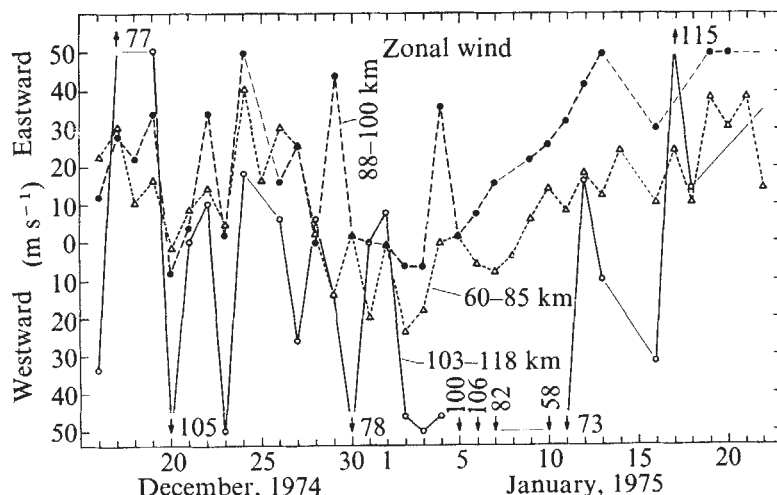
Received August 18; accepted September 22, 1975.

- 1 Rast, N., and Grant, R. H., *Am. J. Sci.*, **273**, 572 (1973).
- 2 Williams, H., Kennedy, M. J., and Neale, E. R. W., *Spec. Pap. geol. Ass. Can.*, **181** (1972).
- 3 Kennedy, M. J., Phillips, W. E. A., and Neale, E. R. W., *24th int. geol. Congr.*, **516** (1972).
- 4 Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **76**, 3179 (1971).
- 5 Upadhyay, H. D., Dewey, J. F., and Neale, E. R. W., *Proc. geol. Ass. Can.*, **24**, 27 (1971).
- 6 Church, W. R., and Stevens, R. K., *J. geophys. Res.*, **76**, 1460 (1971).
- 7 Williams, H., and Malpas, J. G., *Can. J. Earth Sci.*, **9**, 1216 (1972).
- 8 Dewey, J. F., in *The Geology of Continental Margins* (edit. by Burk, C. A., and Drake, C. L.), 933 (Springer, New York, Heidelberg, Berlin, 1974).
- 9 Williams, H., Malpas, J. G., and Comeau, R., *Geol. Surv. Pap. Can.*, **72-1A**, 14 (1972).
- 10 Wilson, J. T., *Nature*, **211**, 676 (1966).
- 11 Hood, P. J., and Bower, N. E., *Geol. Surv. Pap. Can.*, **71-23**, 573 (1973).
- 12 Laughton, A. S., *Nature*, **232**, 612 (1971).
- 13 Davidson, A., *Spec. Pap. geol. Ass. Can.*, **11**, 381 (1972).

Mesospheric and lower thermospheric wind changes over Canada during the January 1975 stratospheric warming

EVIDENCE indicates that the mesosphere and lower thermosphere may be perturbed when stratospheric temperatures increase rapidly in winter. Much of this evidence is indirect, due to limitations inherent in existing techniques of observation, and typically comprises measurements of ionospheric

Fig. 1 Zonal component of daily median wind, for one hour around noon, and in the altitude ranges 60–85, 88–100 and > 103 km, at Saskatoon.



conditions, such as absorption in the lower ionosphere or heights of isoelectronic surfaces up to the E-region. Changes in these conditions are found to be significantly correlated with increases in stratospheric temperatures in winter at latitudes above $\sim 40^\circ$.

There seem to be at least two related classes of stratospheric temperature changes. The first is an aspect of a travelling planetary wave—changes in the mesospheric electron densities associated with such waves have been observed in both the Southern Hemisphere¹, and Northern Hemisphere^{2,3}. The second, which is usually called a stratospheric warming or a major warming, is the reversal, in winter, of the polar vortex to westward flow. It is thought to be initiated by the interaction of a travelling planetary wave with a standing wave^{4,5}. Ionospheric changes related to major warmings have been identified in the Northern Hemisphere⁶. In the Southern Hemisphere, the interaction of travelling and standing planetary waves may not occur to the same extent as in the Northern Hemisphere.

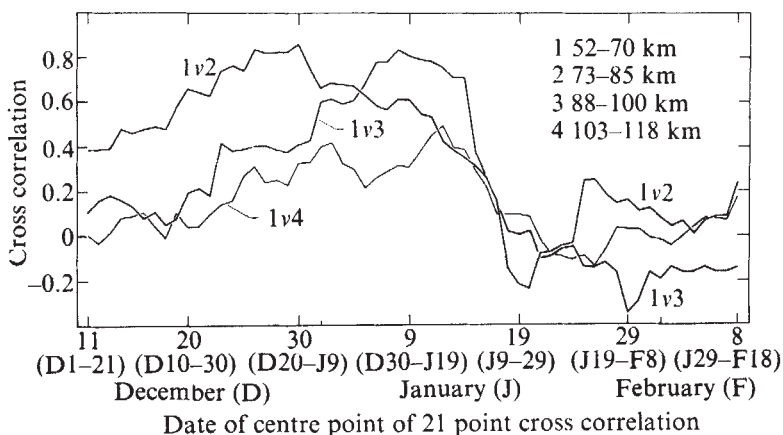
Observations of the winds and temperatures of the mesosphere and lower thermosphere during stratospheric warmings are relatively few. Significant correlations between the temperatures at 30 km and the pressures at 55 km for Fort Greely, Alaska, and between temperatures at 30 km and pressures at 70 km for Wallops Island, Virginia⁷ have, on occasion, been found. A reversal of zonal flow between 75 and 105 km, from eastward to westward, has also been observed in Alaska by means of the radio meteor technique during the warmings of the winters of 1967–68 and 1970–71 (ref. 8).

In the winter of 1974–75 observations of the winds from 50 to 105 km, were made near Saskatoon (52°N , 107°W) and Ottawa (45°N , 76°W), using the partial radiowave

reflection, drift-of-diffraction-pattern technique⁹. Together with meteorological rocket measurements from Churchill (58°N , 94°W), these provide more detailed wind data with better continuity in the vertical direction, than previous experiments provided. The Churchill data (not shown) establish that a major warming was detectable around December 17, when the wind at altitudes between 30 and 40 km began to shift from eastward through southward, to a reversed (or westward) flow, which prevailed on January 9. On those dates, temperatures at altitudes between 30 and 40 km were $\sim -60^\circ\text{C}$ and $\sim -30^\circ\text{C}$ respectively. The flow remained westward until about January 17. For comparison, Fig. 1 shows the behaviour of the zonal component of winds at Saskatoon in three altitude ranges: 60–85, 88–100, and ≥ 103 km. (The height at which total reflection of radio waves at 2.2 MHz is observed, occurs in the latter range.)

Five-year mean values (J. B. Gregory and A. H. Manson, unpublished), have established that the zonal component is normally eastwards up to ~ 95 km at this time of year, in the absence of perturbations caused by warmings. Above 95 km, the flow is normally westwards, and a westward component of the thermal wind, corresponding to a warm high-latitude mesosphere, also normally exists above 75 km during this part of the winter at Saskatoon. Figure 1 shows two forms of departure from this. The first is a short term variability, over intervals of 1–4 d, and is greatest above 100 km. This variability is believed to be due to the effect of gravity waves ($\tau \sim 60$ min) when, because of incomplete data, they are not removed by height and time averaging. The second departure, evident between about December 30 and about January 12, is of interest here, since it coincides with the warming observed at Churchill.

Fig. 2 Cross-correlation coefficients of zonal component of winds at Saskatoon, between four altitude ranges 52–70, 73–85, 88–100 and 103–118 km.



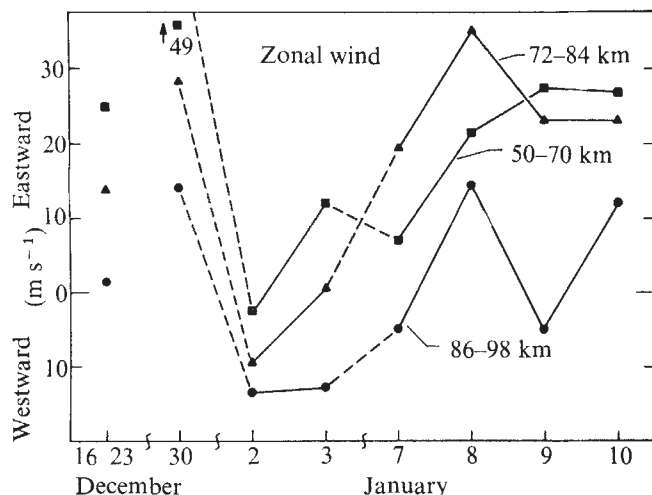


Fig. 3 Zonal component of daily median wind, around noon, in the altitude ranges 50–70, 72–84 and 86–98 km, at Ottawa: (Note the breaks in the sequence of dates.)

By January 1, the zonal wind below 95 km had reversed (and now blew to the west), and the flow above 95 km also altered significantly (Fig. 1). By January 5, recovery had commenced, though to reach speeds lower than before the warming. (A second warming was observed in early March, but is not discussed here.)

The extent to which wind changes at various altitude intervals are related, is demonstrated in Fig. 2. From the daily sequence of observations at Saskatoon, 21-d sequences have been selected, each starting a day after the previous sequence. Cross-correlation coefficients have been computed between the lowest altitude interval, 52–70 km, and successively higher intervals, for each 21-d sequence, with zero relative delay. (Coefficients of value 0.41 are significant at the 5% level, and those of value 0.53 at the 1% level.) The sequence of correlation coefficients shows that coupling existed between altitude intervals up to 85 km by about December 17, and to 100 km around the period of maximum stratospheric change, January 9–17.

Observations at Ottawa also show the effects of the warming. For present purposes, only those observations between December 30 and January 10 are considered. Figure 3 shows the mean zonal speeds, in three height ranges, 50–70, 72–84 and 86–98 km, derived from 40-min records around noon. This figure shows that reversal of the zonal flow, 50–98 km, occurred between December 30 and January 2, and that the initial flow was not resumed until January 8. These dates agree substantially with those for Saskatoon and Churchill.

Our observations, together with others at Saskatoon for the winters 1969–74, and the Alaskan observations⁸, show that changes in mesospheric and lower thermospheric winds can be expected during stratospheric warmings, as can, presumably, temperature changes. Aeronomical changes are undoubtedly a consequence of these dynamical changes. In future studies, simultaneous observations of both dynamical and aeronomical conditions, on a spatial scale adequate to establish transport effects, will be required.

We are grateful to Dr R. S. Quiroz, National Meteorological Centre, for early access to Churchill meteorological rocket data.

J. B. GREGORY
A. H. MANSON
D. G. STEPHENSON

Institute of Space and Atmospheric Studies,
University of Saskatchewan,
Saskatoon, Canada

J. S. BELROSE

M. J. BURKE

T. N. R. COYNE

Communications Research Centre,
Ottawa, Canada

Received June 23; accepted September 25, 1975.

- ¹ Gregory, J. B., and Manson, A. H., *J. atmos. terr. Phys.*, **32**, 837–852 (1970).
- ² Cavalieri, D. J., and Deland, R. J., *J. atmos. terr. Phys.*, **37**, 297–310 (1975).
- ³ Kawahira, K., *Spec. Contrib. Geophys. Inst. Kyoto Univ.*, **10**, 35–47 (1970).
- ⁴ Matsumo, T., *J. atmos. Sci.*, **28**, 1479–1494 (1971).
- ⁵ Quiroz, R. S., *J. atmos. Sci.*, **32**, 211–224 (1975).
- ⁶ Belrose, J. S., *Nature*, **214**, 660–664 (1967).
- ⁷ Heard, W. C., *Nature*, **217**, 1038–1040, (1963).
- ⁸ Hook, J. L., *J. geophys. Res.*, **77**, 3856–3868 (1972).
- ⁹ Manson, A. H., Gregory, J. B., and Stephenson, D. G., *J. atmos. terr. Phys.*, **35**, 2055–2067 (1973).

Cavitation damage in dilute polymer solutions

HERE we report that the addition of small quantities of high molecular weight, water-soluble polymers to water (in our case polyacrylamide) increases the rate of vibratory cavitation erosion of copper. This result, together with that of Kudin¹, who obtained an enhanced rate of jet cutting after a similar addition, strengthens the analogy drawn between the two processes, and indicates that microjets may play a significant role in cavitation damage. These results may well be relevant for rain erosion, a process often compared with cavitation and jet cutting.

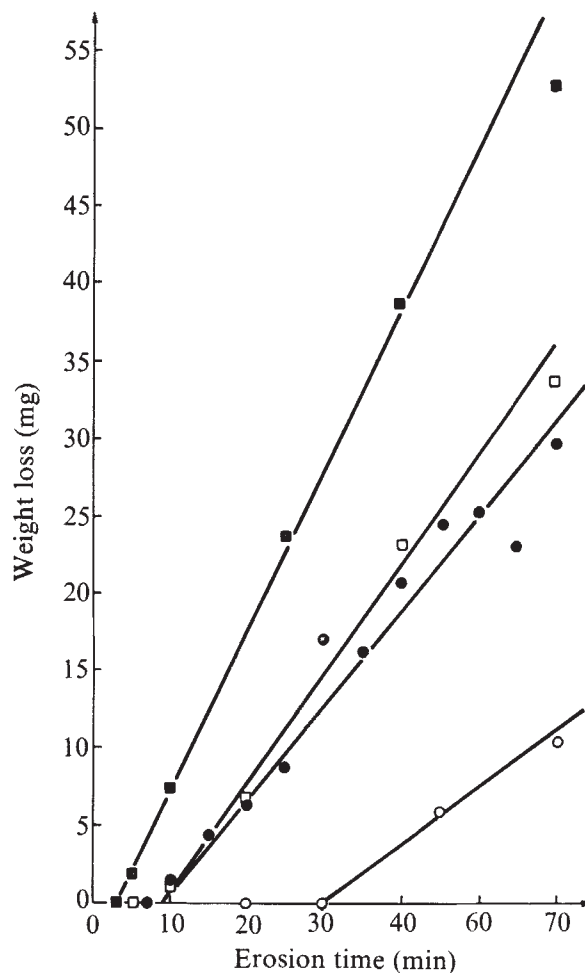


Fig. 1 Curves of vibratory cavitation erosion weight loss as a function of time for copper specimens in: ●, distilled water; □, equilibrated 100 w.p.p.m. polyacrylamide solution; ■, equilibrated 1,000 w.p.p.m. polyacrylamide solution; ○, 2:3 v/v glycerol-distilled water mixture.

Table 1 Experimental details

Solution	Conductivity (μS) (1 Siemen = 1 mho)	pH	Viscosity (cpoise)	Surface tension (dyne cm^{-1})
Distilled water	3.0	5.5	0.89	72.5
Fresh 100 w.p.p.m. polymer solution	8.7	5.5	2.14	72.7
Equilibrated 100 w.p.p.m. polymer solution	9.7	—	1.31	—
Fresh 40 w.p.p.m. polymer solution*	—	—	1.29	—
Fresh 1,000 w.p.p.m. polymer solution	—	—	(26)	73.5
Equilibrated 1,000 w.p.p.m. polymer solution	4.5	5.1	3.85	71.0
Fresh 200 w.p.p.m. polymer solution*	—	—	4.05	—
Glycerol distilled water mixture (2:3, v/v)	10.5	6.0	3.99	68

*These data are included for comparison purposes, to give an indication of the extent of polymer degradation, in terms of loss of viscosity.

Kudin's result¹ seemed to be of particular relevance to the mechanism of cavitation erosion of metals, since one model suggests that damage occurs as the result of the asymmetric collapse of cavities, leading to the formation of high velocity microjets which impinge on, and erode the metal surface (for example, refs 2 and 3). In view of their effects on jet cutting efficiency, it would therefore be anticipated that the presence of these polymers in a cavitating liquid would result in increased cavitation erosion. It has been shown in water tunnel experiments of Ting⁴, however, that polyethylene oxide in tap water inhibits the inception of cavitation. Although this result does not give any indication of the effect on the rate of erosion once cavitation is produced, he⁴ further suggests that, after the onset of cavitation, the asymmetric collapse of cavities and the formation of liquid microjets will also be retarded. It is postulated that this would be the result of increased resistance to flow, created by the viscoelasticity of the polymer solution⁵. If this latter suggestion is correct, it would be anticipated that the addition to cavitating liquids of soluble polymers, with appropriate viscoelastic properties, would result in decreased cavitation erosion.

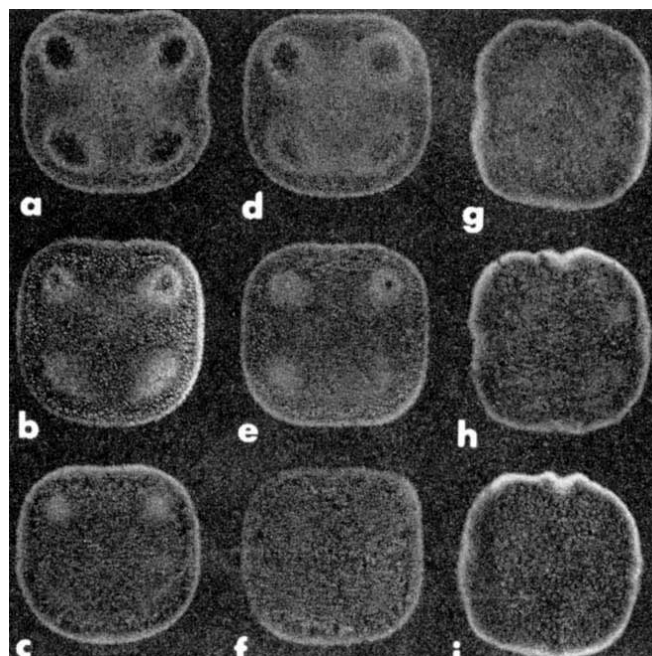
It should be noted that an alternative model suggests that cavitation erosion is caused by the shock waves which result from the approximately symmetrical collapse of bubbles. It can be shown, however, that any increase in viscosity should have a damping effect on the rates of cavity growth and collapse. This would tend to reduce the magnitude of the shock wave, and to result in a decreased rate of cavitation erosion.

The cavitation experiments were undertaken on cold-drawn OFHC copper; the specimens used were disks, 19 mm in diameter and 6.4 mm thick, which were mechanically polished with 6 μm diamond paste. The tests were carried out in distilled water, and in solutions containing 100 w.p.p.m. and 1,000 w.p.p.m. of a water-soluble polyacrylamide (Percol 139, an anionic copolymer of acrylamide and 5% acrylic acid) with a mean molecular weight of about 10^7 . Cavitation was produced by an ultrasonic probe (frequency 20 kHz) positioned immediately above the stationary copper specimen. In all tests, the titanium tip of the probe was 35 mm below the surface of the test solution, the amplitude of vibration was 60 μm , and the separation was 1.3 mm. The bulk temperature of the test solution was maintained at 298 ± 1 K, but no attempt was made to exclude air.

Preliminary experiments showed that the polymer in solution was partially degraded by extensive exposure to cavitation. A continuously refreshed liquid system, with a flow rate of $10^{-4} \text{ m}^3 \text{ min}^{-1}$ into a test vessel with a capacity of $2 \times 10^{-3} \text{ m}^3$, was therefore used. By this means an 'equilibrium' solution, with a constant viscosity, was obtained. Before the start of each cavitation run, the initial solution in the test vessel was degraded to the equilibrium viscosity by means of cavitation in the absence of the copper specimen. The viscosities of the polymer solutions used, before and after equilibration, are reported in Table 1.

Figure 1 shows the cavitation erosion weight loss-time curves for copper in distilled water, and in equilibrated solutions containing 100 w.p.p.m. and 1,000 w.p.p.m. polyacrylamide. With the exception of relatively long exposure times, the effect of

Fig. 2 Surface appearance of copper specimens after vibratory cavitation erosion in: *a*, distilled water for 10 min; *b*, distilled water for 20 min; *c*, distilled water for 60 min; *d*, equilibrated 100 w.p.p.m. polyacrylamide solution for 10 min; *e*, equilibrated 100 w.p.p.m. polyacrylamide solution for 20 min; *f*, equilibrated 100 w.p.p.m. polyacrylamide solution for 60 min; *g*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 10 min; *h*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 20 min; *i*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 60 min. Magnification $\times 2.2$.



100 w.p.p.m. polymer additions is negligible. It is clear, however, that in the case of 1,000 w.p.p.m. polymer additions, the incubation time is reduced from ~ 8 to ~ 3 min, and the rate of weight loss is increased by a factor of ~ 1.4 . These quantitative observations are confirmed qualitatively by the surface appearance of the specimens after erosion for equivalent times in the three liquids, shown in Fig. 2.

The data presented in Table 1 indicate the extent to which the polymer additions increase the viscosity of distilled water. It is widely accepted that, in the absence of other overriding factors, an increase in viscosity of the cavitating liquid results in less severe cavitation damage^{6,7}. This effect has been confirmed for the present experimental conditions by undertaking cavitation tests on pure copper in a 2:3 (v/v) glycerol-distilled water mixture, which has a viscosity comparable with that of an equilibrated 1,000 w.p.p.m. polymer solution. The results of these experiments are also shown in Fig. 1. The increase in viscosity associated with the addition of glycerol to distilled water, increased the incubation time to ~ 30 min, and decreased the erosion rate by a factor of ~ 0.6 compared with that observed in distilled water. These data clearly confirm that the increased damage associated with polyacrylamide additions cannot be due to an increase in viscosity.

The data shown in Table 1 also indicate that the addition of soluble polyacrylamide does not significantly affect the surface tension of distilled water. The importance of these data lies in the fact that it has been shown that a marked decrease in surface tension, produced by the addition of a detergent, can also increase cavitation damage (V. A., D. Hulse, and R. P. M. P., unpublished).

Our result with additions of 1,000 w.p.p.m. polymer is in direct contrast to the theoretical predictions of Ting⁴, but is fully consistent with the observed effect of such additions to jet cutting solutions.

The above results give no indication as to the mechanism by which the addition of polyacrylamide increases cavitation damage. It should be noted that, although the polyacrylamide does not significantly affect the surface tension of the solutions, it does increase markedly their viscosity. It has been shown experimentally, however, that an equivalent increase in viscosity, produced by the addition of glycerol, greatly decreases cavitation damage. Clearly, whatever the mechanism by which polyacrylamide additions increase cavitation damage, it is sufficient, in the case of the 1,000 w.p.p.m. solution, to outweigh the decrease in cavitation damage which would be expected on the grounds of viscosity alone. In the case of the 100 w.p.p.m. solution, it is possible that the two opposing effects balance each other.

V. ASHWORTH
R. P. M. PROCTER

Corrosion and Protection Centre,
University of Manchester Institute of Science and Technology,
PO Box 88, Manchester, UK

Received August 7; accepted September 30, 1975.

¹ Kudin, A. M., *et al.*, *Nature*, **245**, 95 (1973).

² Mitchell, T. M., and Hammitt, F. G., *Trans. Am. Soc. mech. Engrs, J. Fluid Engng*, **95**, 29 (1973).

³ Kling, C. L., and Hammitt, F. G., *Trans. Am. Soc. mech. Engrs, J. bas. Engng*, **94**, 825 (1972).

⁴ Ting, R. Y., *U.S. Nat. Bur. Standards, Spec. Publ.* **394**, 100 (1974).

⁵ Ting, R. Y., *J. appl. Polym. Sci.*, **16**, 3169 (1972).

⁶ Plesset, M. S., *Trans. Am. Soc. mech. Engrs, J. bas. Engng*, **92**, 807 (1970).

⁷ Wilson, R. W., and Graham, R., *Proc. Conf. on Lubric. and Wear* (Inst. mech. Eng 1957).

Anomalous thermal behaviour of induced phosphorescence in irradiated, doped polymeric glasses

WE report here that the intensity of induced phosphorescence in pre-irradiated, doped polymethylmethacrylate (PMMA) glasses changes in an anomalous way with temperature. At room temperature the irradiated part of the

sample shows intense phosphorescence after ultraviolet excitation with an inspection lamp¹, whereas the part of the sample not previously irradiated shows no afterglow. When, however, the sample is cooled to the temperature of liquid nitrogen and the ultraviolet photoexcitation is repeated, the irradiated spot appears dark on the bright afterglow background of the rest of the sample (Fig. 1a and b). In Fig. 1a the central, ultraviolet irradiated, spot is seen as a bright

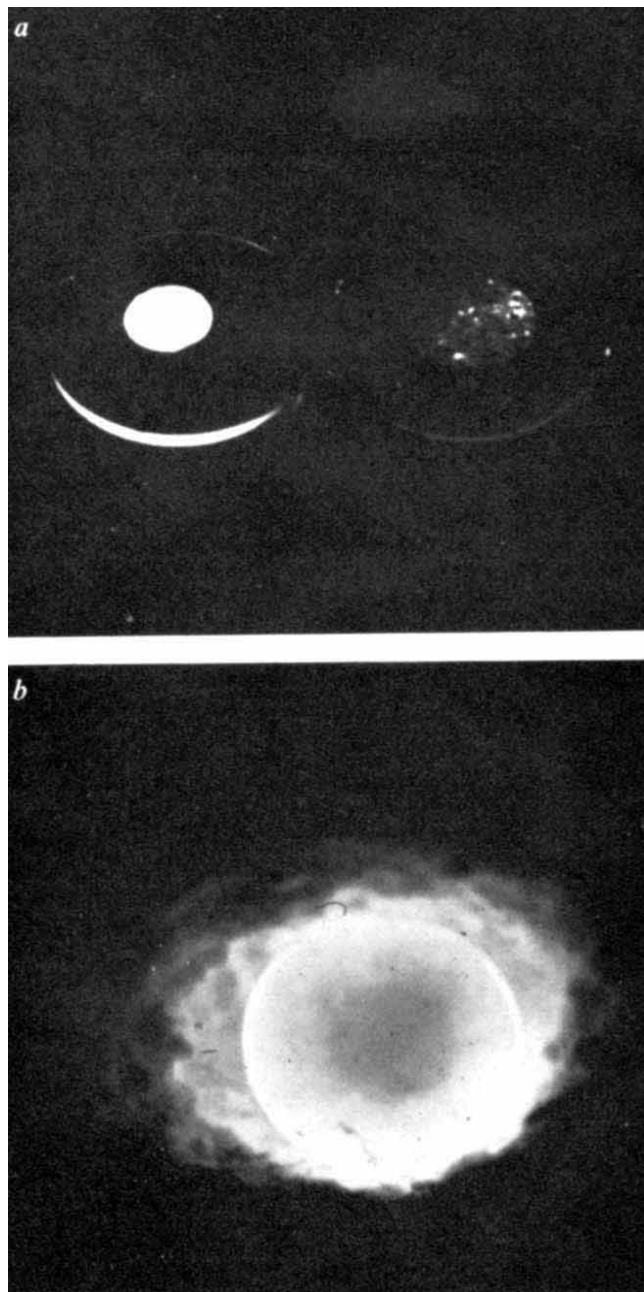


Fig. 1 a, Phosphorescence at room temperature after ultraviolet excitation has been switched off: left hand side, an X-ray irradiated sample; right hand side, an ultraviolet irradiated sample. X-ray and ultraviolet irradiated regions show clearly emission in the central areas of the samples; some of the emission is transmitted to the edges by a light guiding effect of the plane surfaces of the samples. The regions in the outer portion of the samples were not pre-irradiated and do not show room temperature luminescence. b, Phosphorescent glow in sample on the right of Fig. 1a, after it had been immersed in liquid nitrogen and the ultraviolet excitation had been switched off. The central area, which had been pre-irradiated with ultraviolet radiation, shows less phosphorescence than the area which had not been irradiated. As in Fig. 1a some light leakage is seen around the rim of the sample and in the liquid nitrogen.

circular region at room temperature after excitation with an ultraviolet inspection lamp; Fig. 1b shows the same irradiated spot, now relatively dark, after the sample has been cooled to 77 K and excited by the same inspection lamp.

Triphenylene is a convenient dopant because of its long triplet lifetime, 16 s, compared with dibenzothiophene which has a shorter lifetime of 1.5 s. Samples containing coronene as the dopant in PMMA after ultraviolet irradiation also show similar photographic results at room temperature and at low temperatures; visual inspections, using several dopants including dipbenzothiophene, dibenzothiophene sulfone and dibenzofuran have confirmed this. Quantitative studies have also confirmed that the induced phosphorescence shows an anomalous temperature-intensity relationship. The variation in the peak intensity of phosphorescence (measured at 462 nm) over a range of temperatures down to 188 K, for samples of non-irradiated polymethylmethacrylate doped with dibenzothiophene is shown in Fig. 2a. The curve is typical of those obtained for a number of different samples; because of variations in the thermocouple position slight shifts have been observed in the curves. The curves exhibit behaviour typical of normal

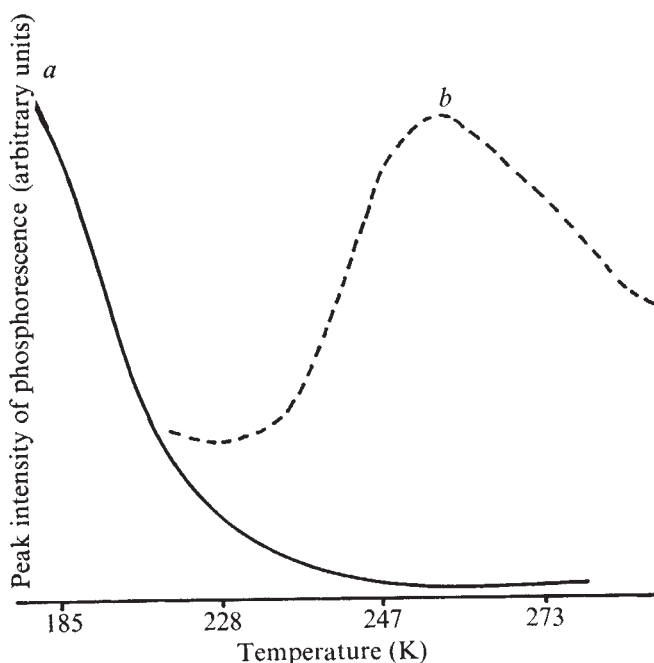


Fig. 2a. Peak intensity of phosphorescence against temperature for a non-irradiated sample of doped PMMA; b, induced intensity of phosphorescence against temperature for an irradiated, doped sample of PMMA.

phosphorescence in doped, organic glass matrices². The accepted explanation of this effect is that as the temperature of the system is lowered the probability that non-radiative deactivation of the lowest triplet state (T_1) to the lowest state (S_1) will occur is reduced. This reduction is much greater than the reduction of energy transfer between higher singlet levels or between the first excited singlet state and the lowest triplet state. There is, therefore, still an appreciable energy transfer taking place between S_1 and T_1 so as to enable a certain number of triplet levels to be populated. These will then be emptied, increasingly, by radiative transitions, and thus phosphorescence is observed.

The behaviour of the induced phosphorescence peak of ultraviolet irradiated polymethylmethacrylate doped with dibenzothiophene is shown in Fig. 2b. Curves from a number of similar experiments show slight shifts in

maxima; this is probably caused by variations in the position of the thermocouple with respect to the emitting surface. The main distinctions between Fig. 2a and b are, first, a non-irradiated sample does not show room temperature phosphorescence, whereas an irradiated sample shows an appreciable intensity of phosphorescence at room temperature; second, unlike the non-irradiated sample, the intensity of phosphorescence of the irradiated sample does not continue to rise as the temperature is reduced; it shows a maximum in the region of 250 K, after which the intensity falls as the temperature is further reduced. At about 228 K the intensity of phosphorescence of the irradiated sample falls to a value that is about the same as that of the non-irradiated sample. Further it does not show a marked increase as the temperature continues to be reduced to 185 K. This is contrary to the behaviour of non-irradiated samples.

A full explanation of this behaviour cannot be given at present; however, some of the features can be explained. Three temperature regions can be identified. Region I, from room temperature down to approximately 240 K, starts with significant phosphorescence at room temperature and increases to a maximum. We have postulated^{1,3,4} that radicals or traps derived from PMMA, are involved in energy transfer from PMMA to the triplet state and that there is no exclusive requirement for population through the excited singlet state of the dopant. If it is assumed that all non-radiative processes diminish as the temperature is decreased, then curve b (region I) in Fig. 2 is consistent with the usual temperature behaviour for phosphorescence in photoinert matrices. The intensity in PMMA, moreover, is very substantially higher because of direct population of the triplet state by energy transfer. In temperature region II, from approximately 240 K down to approximately 230 K, the induced phosphorescence decreases sharply, in contrast with the behaviour for a photoinert matrix. Assuming, as is reasonable, that the rates of the radiative processes are unaffected, but that the non-radiative decay processes are slightly decreased, it seems that energy transfer between the PMMA radicals and the triplet levels is involved.

The energy transfer which seems to diminish considerably in region II may be affected, possibly by the progressive freezing out of the diffusional mobility of the radicals or, more probably, by a reduction in the extent of the appropriate geometrical orientation of energy transfer partners. PMMA is known to have a γ transition at 268 K, attributed to hindered rotation of the ester group⁵. Temperature range III, below 230 K, shows a slight increase in phosphorescence. It can be assumed that energy transfer has been reduced to a minimum and the increase would then correspond to the further reduction of non-radiative processes from the triplet level. The intensity then lies below that for a photoinert matrix and much of the input energy is therefore degraded in the matrix (Fig. 2).

The enhancement of phosphorescence at room temperature is a striking and useful phenomenon though it does not persist below 230 K in this particular system.

C. S. BILEN
D. J. MORANTZ

London College of Printing,
Elephant and Castle,
London SE1 6SB, UK

Received August 5; accepted August 21, 1975.

- Morantz, D. J., and Bilen, C. S., in *Reactivity of Solids* (edit. by Anderson, J. S., Roberts, M. W., and Stone, F. S.), 525-535 (Chapman and Hall, London, 1972).
- Meyer, B., *Low Temperature Spectroscopy*, 65-104 (Elsevier, New York, 1971).
- Morantz, D. J., and Bilen, C. S., *Polymer*, 16, 745-748 (1977).
- Morantz, D. J., and Irons, A. C. M., in *Transitions non-Radiatives dans les Molecules*, 158-162 (Société de Chimie Physique, Paris, 1969).
- Wüdrich, K., *J. Polym. Sci., Pt A-2*, 12, 201-212 (1974).

Measurements of erythemally effective ultraviolet radiation and total ozone content

MEASUREMENTS of erythemally effective ultraviolet radiation—wavelengths between 290 and 320 nm—are now being made at Aspendale (38°S, 145°E) and Brisbane (27°S, 153°E) as part of separate studies being undertaken by research groups in Australia and the USA. The following preliminary analysis of these measurements supplements the theoretically calculated relationships¹⁻⁴ between the total atmospheric ozone content and the intensity of ultraviolet radiation incident at the Earth's surface.

The data were obtained at Aspendale between May 1966 and February 1970, using an instrument described by Robertson⁵, and between June and August 1974 with two instruments—one described by Urbach *et al.*² and one developed by CSIRO; the

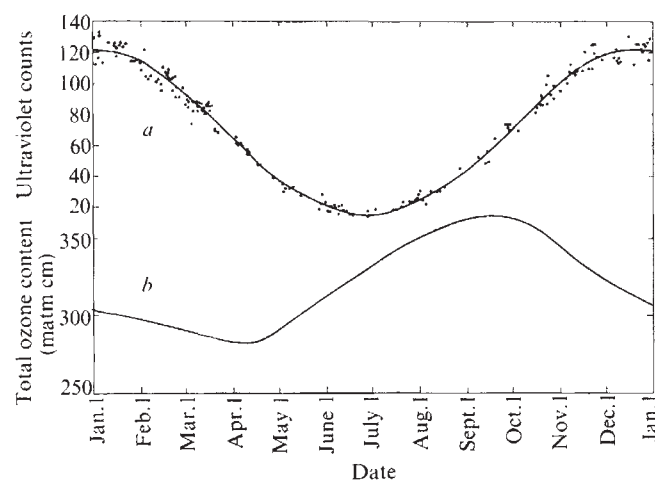


Fig. 1 *a*, Maximum half-hourly ultraviolet total for clear days at Aspendale, May 1966–February 1970. *b*, Mean annual variation of total ozone content at Aspendale.

Brisbane data were obtained between May and July 1974, also using the instrument of Urbach *et al.* The three instrument types are similar in operation, and have almost identical spectral responses. Each unit prints the half-hourly totals of integrated erythemal effectiveness of the ultraviolet radiation. The present analysis was performed for cloudless days only, and these days were selected from continuous records of global and diffuse short-wave radiation at Aspendale, and from visual reports of cloud cover at Brisbane. For each set of data listed in Table 1, a graph showing the daily maximum half-hourly total of ultraviolet radiation (the solar noon ultraviolet irradiance) was plotted against time, and a line of best fit drawn by eye. The graph for Aspendale is shown in Fig. 1*a*. In addition, graphs were plotted of the mean annual variation of total ozone content, from eighteen years of data for both Aspendale and Brisbane. The Aspendale curve is shown in Fig. 2*b*. The departures of both total ozone content and the noon ultraviolet irradiance from their mean values, were then expressed as percentages, and plotted against each other as in the example of Fig. 2 for Aspendale 1966–70. A line of best fit was computed using a

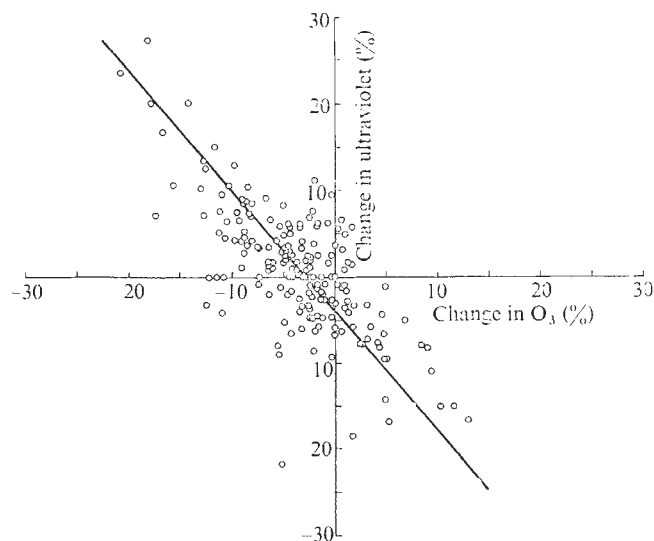


Fig. 2 Percentage change of ultraviolet intensity from the mean value as a function of percentage change of ozone content from the mean value. The graph is for the Aspendale (1966–70) data. —, Line of best fit.

method of least squares, in which the ozone and ultraviolet measurements were assumed to have similar relative errors⁶. For each set of data, the slope of the line of best fit is a measure of the change in the ultraviolet irradiance, which results from a change in total ozone content. These measured dependences are given in Table 1.

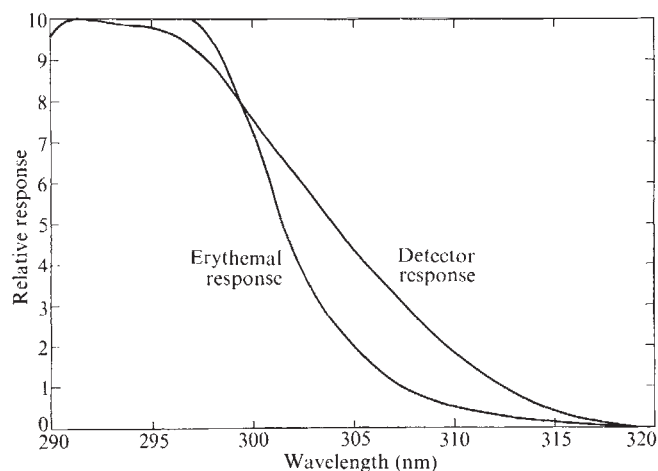


Fig. 3 The spectral response of the detectors, and the erythemal response of human skin.

In the table, the measured dependences are compared with those calculated using the equations and semi-empirical parameters of Green *et al.*⁷. These equations and parameters, which are based on the results of Bener (unpublished) give the ultraviolet irradiance at individual wavelengths in the range 280 to 340 nm as a function of solar zenith angle and total ozone content. The expected increase in 'measured' irradiance, for a total

Table 1 Results of clear day analysis

Location	Period	Instrument	No. of cloudless days	Ultraviolet dependence on total ozone content*		
				Measured	Calculated Detector	Erythemal
Aspendale	May 66–Feb. 70	Robertson ⁵	184	–1.40	–1.4	–1.7
Aspendale	June 74–Aug. 74	CSIRO	18	–1.32	–1.6	–1.8
Aspendale	June 74–Aug. 74	Urbach <i>et al.</i> ²	22	–1.36	–1.6	–1.8
Brisbane	May 74–July 74	Urbach <i>et al.</i> ²	31	–1.03	–1.4	–1.6

*The figures given are the ratios of percentage change in ultraviolet radiation intensity to percentage change in total ozone content.

ozone decrease of 10%, was then obtained by combining the calculated spectral irradiance with the spectral response of the detectors (Fig. 3). In addition, the dependences of erythemally effective ultraviolet irradiance on the total ozone content were calculated as above, but using the actual erythral response curve given in Fig. 3. For the Aspendale 1966–70 data there is excellent agreement between the measured and calculated values. The other three periods of measurement give less satisfactory agreement, presumably because of the limited amount of data.

The Australian monitoring network now includes four stations, and further stations are planned. The importance of such long term monitoring programmes in this band of radiation is shown by the recent concern regarding the effects of possible anthropogenic modification of the stratospheric ozone layer (see, for example, ref. 8).

I. J. BARTON

CSIRO Division of Atmospheric Physics,
Aspendale, Victoria 3195

D. F. ROBERTSON

University of Queensland,
St Lucia, Queensland 4067, Australia

Received July 21; accepted September 30, 1975.

- ¹ Bassett, I. M., Box, M. A., and Hewitt, R. G. L., *Search*, **5**, 182–186 (1974).
- ² Urbach, F., Berger, D., Robertson, D. F., and Davies, R. E., in *Proc. third CIAP Conf.*, Cambridge, Massachusetts (1974).
- ³ Cutchis, P., *Science*, **184**, 13–19 (1974).
- ⁴ Schulze, R., in *Proc. Int. Conf. Struct. Composition and General Circulation of the Upper and Lower Atmospheres and Possibly Anthropogenic Perturbations*, 479–493, Melbourne, Australia (1974).
- ⁵ Robertson, D. F. in *The Biologic Effects of Ultraviolet Radiation* (edit. by Urbach, F.), 433–436 (1969).
- ⁶ Morgan, W. A., *Q. Jl. R. met. Soc.*, **86**, 107–113 (1960).
- ⁷ Green, A. E. S., Sawada, T., and Shettle, E. P., *Photochem. Photobiol.*, **19**, 251–259 (1974).
- ⁸ Molina, M. J., and Rowland, F. S., *Nature*, **249**, 810 (1974).

Movement of the hyoid apparatus during chewing

THE movements of the hyoid bone and the larynx during swallowing are only well established for man^{1–3}. Their movements, if any, during mastication in man and in other mammals have been neglected. The hyoid is suspended from the cranium, above and behind, by the posterior suprahyoid muscles (stylohyoid and the posterior belly of digastric) and from the symphyseal region of the jaw in front by the anterior suprahyoid muscles (anterior belly of digastric, mylohyoid, geniohyoid and the more inferior fibres of genioglossus). The infrahyoid, or 'strap' muscles of the neck connect the hyoid with the sternum directly by the sternohyoid and indirectly through the thyroid cartilage of the larynx (thyrohyoid and sternothyroid) as well as with the scapula (omohyoid). This arrangement of the hyoid apparatus is found in all mammals, except that the digastric may not have a direct hyoid connection⁴: a situation occasionally found in man². These three groups of muscles (anterior and posterior suprahyoids and infrahyoids) are recognised, on the basis of anatomical, cinematographic⁵ and electromyographic⁶ studies of human deglutition, as acting in combination first to elevate and then to depress the hyoid and larynx in swallowing. The posterior suprahyoids and the infrahyoids are also classically regarded^{1,2} as acting to 'stabilise'¹ or 'fix'² the hyoid so that the anterior suprahyoids, and particularly the anterior belly of digastric^{6,7}, can pull the symphyseal region of the jaw towards the hyoid and open the mouth. Mandibular depression follows the power stroke⁸ of every chewing cycle. The classical view of the hyoid apparatus is of a somewhat immobile bone and a group of muscles whose only active role is in swallowing. This concept may have led to the view that, since the omohyoid in man made such an uncertain contribution to hyoid movement or control of hyoid position, a 'specific'¹¹

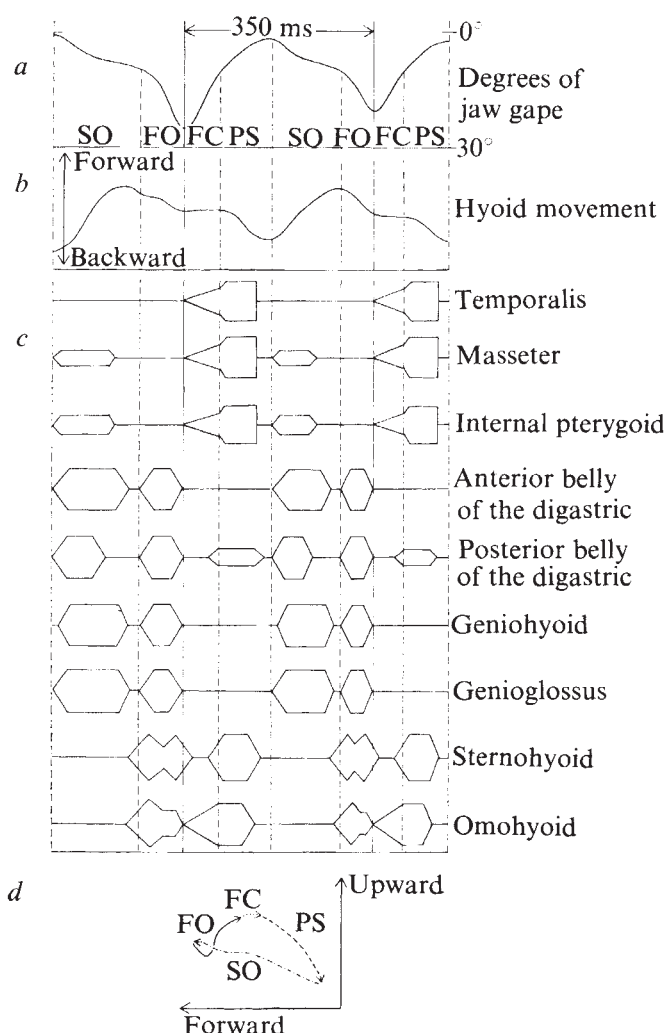


Fig. 1 Jaw and hyoid movement (*a*, *b*) in the opossum during part of a typical sequence of chewing on soft food. In *a*, the relative movement of upper and lower jaws has been plotted as degrees of gape (vertical axis) against time (horizontal axis). Each chewing cycle has four stages: FC, fast closing; PS, power stroke, (in this case consisting almost entirely of a slow closing movement); SO, slow opening; and FO, fast opening. In *b* the movements of the hyoid bone have been plotted on a plane parallel to the secondary palate; the range of antero-posterior movement is of the order of 20 mm. The associated EMG activity in adductor and representative hyoid muscles is shown in *c* as the envelope of the electrical signal. *c*, Composite diagram based on data from a large number of experiments. The path of movement of a point on the hyoid during chewing is shown in *d*. The diagram was obtained by plotting the position of the point in the 'vertical' and 'horizontal' planes (with reference to the hard palate, see text) at equal intervals of time during the cycle shown in *a*. Small skin incisions were made in anaesthetised animals so that bipolar wire EMG electrodes⁹ could be inserted into the jaw elevators¹⁰ and the major hyoid muscles (both bellies of digastric, geniohyoid, genioglossus, sternohyoid and omohyoid) under direct vision. On recovery, the animals were fed a minced proprietary canned dog food. Cinefluorographic recordings of jaw and hyoid movement were made in the lateral projection at 60 frames s⁻¹ on 16-mm film⁸. EMG activity was recorded simultaneously on magnetic tape, together with a frame-by-frame synchronisation signal from the cine camera. The position of the jaws was measured on each frame of film as degrees of gape¹¹. The position of the hyoid was measured separately in both the vertical and antero-posterior planes: for the former, the palatal line was used as a reference plane and for the latter, a perpendicular from that plane through the squamo-dentary joint. The tape recordings of the EMG activity were then converted into paper records and the positions of both the jaw and the hyoid were plotted on to these records: the data from each frame of film were registered against each synchronisation signal and a movement profile obtained.

and "speculative"² function should be attributed to this muscle; namely the tensing of the cervical fascia to assist prolonged inspiration during forced respiration. The latest edition of *Gray's Anatomy*² comments, "so far no useful data in regard to the hyoid musculature as a whole have been contributed by electromyographers" (page 508).

The American opossum (*Didelphis virginiana*), which is regarded as exemplifying both early mammalian cranio-facial organisation in general and the mammalian hyoid apparatus in particular, has been studied by synchronised cinefluorography and electromyography (see legend to Fig. 1).

Jaw movement in the chewing cycle of the opossum is shown in Fig. 1a. The associated movements of the hyoid in the antero-posterior plane are also shown (b). Movement of the hyoid in both the horizontal and vertical planes is shown in Fig. 1d. The chewing cycle begins with the mouth wide open (maximum gape). At this point the hyoid is about a third of the way along its antero-posterior movement path. As the jaw is elevated rapidly, the hyoid moves very slightly upwards but may not significantly change its antero-posterior position. Both the fast closing movement of the jaw and the elevation of the hyoid cease at tooth-food-tooth contact. As the jaw is moved upwards against the resistance of the food in the 'slow close' of the power stroke, high amplitude EMG activity (Fig. 1c) is seen in the elevator muscles^{12,13}. At the same time, high amplitude activity in the sternohyoid and omohyoid muscles is associated with downwards and backwards movement of the hyoid. No electrical activity was recorded in the anterior suprahyoid muscles during the power stroke. On completion of the power stroke, the jaw has reached its maximum elevation and the hyoid its most inferior and posterior position. The opening stroke of the chewing cycle occurs in two stages; slow opening and then fast opening. In the first, the jaw is moved slowly downwards as the hyoid moves both forwards and upwards. These convergent movements of the jaw and hyoid are associated with activity in the anterior belly of the digastric, the geniohyoid and the genioglossus, all of which are acting to depress the lower jaw and elevate the hyoid simultaneously. There is a small burst of EMG activity in the posterior belly of digastric coincident with the initial early elevation of the hyoid. The sternohyoid and omohyoid are electrically silent during slow jaw opening and do not, therefore, prevent the hyoid from being drawn forwards. Low level activity in the jaw elevators, particularly the masseter and internal pterygoid was recorded during slow opening¹², when the tongue is collecting and repositioning food within the mouth. As the hyoid is moved forwards and upwards, the floor of the mouth and the base of the tongue are also raised by the contracting anterior suprahyoid muscles. The activity in these muscles tends to depress the jaw, which is partially counteracted by the activity in the elevators. Fast opening of the jaw occurs after the hyoid has reached its most anterior and elevated position at the end of slow opening. As the jaw is depressed, the anterior part of the tongue, protruded in slow opening, is retracted sharply. The hyoid is moved slightly upwards and backwards to its midway position. Rapid opening of the jaw is associated with high amplitude EMG activity in all the muscles of the hyoid apparatus.

Contrary to classical anatomical teaching, at no time during the chewing cycle is the hyoid 'fixed'. It shows least movement during the fast close stage of the cycle and most movement during opening. This is not what would be expected were the mandible depressed towards a 'fixed hyoid'. During slow closure in the power stroke, the hyoid moves downwards and backwards; in slow opening it is moved upwards and forwards. There are two important aspects of the pattern of muscle activity responsible for the hyoid cycle. First, the EMG activity in the anterior

and posterior bellies of digastric is asynchronous, both in its time of onset and in its peak amplitude; this finding contrasts with the statement "that (in man) the two bellies must contract with equal force"¹. Both bellies are, however, involved in fast opening when all the muscles of the hyoid apparatus are electrically active and are combining both to depress and retract the hyoid and to depress the lower jaw. Second, during slow opening, the infrahyoid muscles are not actively contracting and do not, therefore, 'stabilise' the hyoid.

In the light of these results, the classical view of the role of the hyoid apparatus must be revised. The hyoid has a cyclical movement completely integrated with that of the jaw and related to the role of the tongue in positioning the food. Further, the hyoid is part of a dynamic and not a basically static system. In man, the acquisition of the upright posture has resulted in some minor shifts in the angulation of the hyoid muscles compared with *Didelphis* but not in any significant change in their anatomy. The initiation and regulation of hyoid movement may, therefore, be seen as the normal function of the omohyoid. This does not, however, preclude the possibility that this muscle, whose anatomy in man has seemed somewhat inexplicable, occasionally assists in tensing the cervical fascia in the specialised respiratory state of prolonged inspiration^{1,2}.

We acknowledge the support of a grant from the USPHS (NIH).

A. W. CROMPTON
PAMELA COOK

Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts 02138

KAREN HIIEMAE*

Unit of Anatomy with special
relation to Dentistry,
Guy's Hospital Medical School,
London SE1 9RT, UK

A. J. THEXTON*

Royal Dental Hospital School of
Dental Surgery,
Leicester Square, London W1, UK

Received July 17; accepted September 24, 1975.

*Also at: Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138.

¹ Last, R. J., *Anatomy, Regional and Applied*, 2nd ed. (Churchill, London, 1959).
² *Gray's Anatomy*, 35th ed. (edit. by Warwick, R., and Williams, P. L.) (Longmans, London, 1973).

³ Carlöö, S., *Acta anat.*, **26**, 81-93 (1956).

⁴ du Chaine, J., *Journ. de l'Anat.*, **50** (1914-1919).

⁵ Whillis, J., *J. Anat. Lond.*, **80**, 115-116 (1946).

⁶ Doty, R. V., in *Handbook of Physiology*, 4 (edit. by Code, C. F.), ch. 92, 1861-1902 (American Physiological Society, Washington DC, 1968).

⁷ Moyers, R. E., *Am. J. Orthodont.*, **36**, 481-515 (1950).

⁸ Crompton, A. W., and Hiimae, K., *Zool. J. Linn. Soc.*, **49**, 21-47 (1970).

⁹ Basmajian, J. V., and Stecko, G., *J. appl. Physiol.*, **17**, 849 (1962).

¹⁰ Hiimae, K., and Jenkins, F. A., *Postilla*, **140**, 1-40 (1969).

¹¹ Hiimae, K., and Kay, R. F., in *Symp. fourth Int. Congr. Primat.* (edit. by Zingales, M. R.), 28-64 (Karger, Basle, 1973).

¹² Thexton, A. J., and Hiimae, K., *ADR abstracts* L378, PL95 (International Association for Dental Research, London, 1975).

¹³ Thexton, A. J., in *Clinical and Physiological Aspects of Mastication* (edit. by Anderson, D. A., and Matthews, B.) (John Wright, Bristol, in the press).

Fate of teratocarcinoma cells injected into early mouse embryos

ANALYSIS of early mammalian development is complicated by technical difficulties. The initial processes of cellular determination and differentiation in the mouse embryo take place in small populations of cells^{1,2}, and major embryogenic events occur after uterine implantation when the embryo is largely inaccessible. Recent work, however, suggests that murine teratocarcinomas may provide a convenient model for studying mammalian development³⁻⁶. These are transplantable tumours of germ cell or embryonic cell origin³⁻⁶, typically consisting of a variety of differentiated tissues and undifferentiated stem cells. The stem cells, called embryonal carcinoma, resemble cells of

Table 1 Details of embryonal carcinoma cell lines

Cell line	Tumour of origin	Chromosomes of tumour		Mouse strain	GPI type	Pigmentation		Approximate number of generations in culture
		Sex	Number			Albino	Agouti	
C86†	88‡ from 8-d embryo	XX	41	C3H	<i>I^b/I^b</i>	+ / +	<i>A/A</i>	4-18
C17†	17‡ from 7-d embryo	XX	40	C3H	<i>I^b/I^b</i>	+ / +	<i>A/A</i>	10-12
SIKR-OSB§	OTT5568 from 3-d embryo	XO*	40*	129	<i>I^a/I^a</i>	+ / +	<i>A^w/A^w</i>	45

*These data refer to the chromosomes of the cultured cells rather than to the tumour of origin.

†M.W. McB. and C.F.G., unpublished.

‡S. A. Iles and E. P. Evans, unpublished.

§Ref. 20.

early embryos in morphological, biochemical and cell surface properties, and in developmental potential³⁻⁶. They can be propagated in tissue culture to provide sufficient material for biochemical analysis. After inoculation into histocompatible adult hosts they form differentiated teratocarcinomas. They also differentiate *in vitro*^{7,8} where the first stages of their differentiation seem to parallel normal embryonic development. We show here that embryonal carcinoma cells can participate in normal embryogenesis, thus providing further evidence for the validity of the use of these cultures as a model of normal embryonic development.

When mouse blastocysts are injected with genetically distinct embryonic cells⁹, they develop normally and tissues of the resulting offspring are often chimaeric, consisting of cells of both donor and host origin. The developmental potential of injected cells can be ascertained from the distribution and

Table 2 Rate of development of blastocysts injected with embryonal carcinoma cells after transfer to females that subsequently became pregnant

Cell line injected	Stage of pseudopregnant foster mother	Foetuses recovered		Number chimaeric
		Embryos transferred	9.5 d Term	
C86	2.5 d	13/18(2)*	—	3(+2?)
SIKR-OSB	2.5 d	—	4/9(1)	1
C17	0.5 d	—	9/13(1)	1
	2.5 d	—	34/58(6)	3
C86	0.5	—	5/12(2)	0
	2.5 d	—	69/93(13)	6

* Figures in parentheses refer to the number of foster mothers to which the embryos were transferred.

genetic activity of their progeny in such chimaeras^{10,11}. Brinster¹² injected cells from the ascitic form of an *in vivo* teratocarcinoma into blastocysts of a different genotype and obtained one overtly chimaeric mouse. The purpose of our study was to confirm and extend this work by injecting cells from *in vitro* cultures of embryonal carcinoma into blastocysts. We obtained normal chimaeric mice following injection of two different embryonal carcinoma cell lines. A third line colonised the embryo but the resultant chimaeric mice developed teratocarcinomas postnatally.

The genetic markers used to distinguish embryonal carcinoma cell progeny from the cells of the host embryo were isozymal differences at the glucose phosphate isomerase (GPI) locus (*Gpi-1*), albino or pigmentation, and white-bellied agouti (*A^w*) or extreme non-agouti (*a^c*). Details of the origin and characteristics of the cell lines are presented in Table 1. Cultured embryonal carcinoma cells derived from C3H teratocarcinomas were injected into blastocysts obtained by matings of *Gpi-1^a/Gpi-1^a* albino CFLP random-bred mice (Anglia Laboratory Animals Ltd). The 129/Sv-*Sl-1*CP derived cell line was injected into *Gpi-1^b/Gpi-1^b* blastocysts obtained from matings of *a^c/a^c* mice of the AG/Cam-derived balanced lethal yellow (*A^y/a^c*) inbred line¹³.

Clumps of 20 to 40 embryonal carcinoma cells were removed mechanically or with trypsin from the culture vessel, and injected into the blastocoelic cavities of 3.5-4-d blastocysts with the aid of a Leitz micromanipulator assembly¹⁴. After 1-3 h

incubation at 37 °C, blastocysts were transferred either to the uterus of day 3 or to the oviduct of day 0 pseudopregnant females. The foster mothers were either killed on day 10 of gestation or allowed to carry their young to term. Chimaerism in postimplantation embryos and internal tissues of offspring was investigated by electrophoretic analysis of homogenates for GPI in starch gels¹⁵. External chimaerism in offspring was determined by inspection of the coat and eyes for the presence of pigmented melanocytes or agouti hairs. Material for histological examination was fixed in neutral formal saline, embedded in wax and sections were stained with haemalum and eosin or Masson's trichrome stain.

In a preliminary series of experiments involving C86 cells, the foster mothers were killed on day 10 of gestation and all conceptuses were separated into a trophoblastic and embryonic fraction. Thirteen normal living embryos were recovered, and no resorption sites were present. GPI analysis indicated the presence of a significant minor component of C86 derived isozyme in three conceptuses: in the embryonic portion of one,

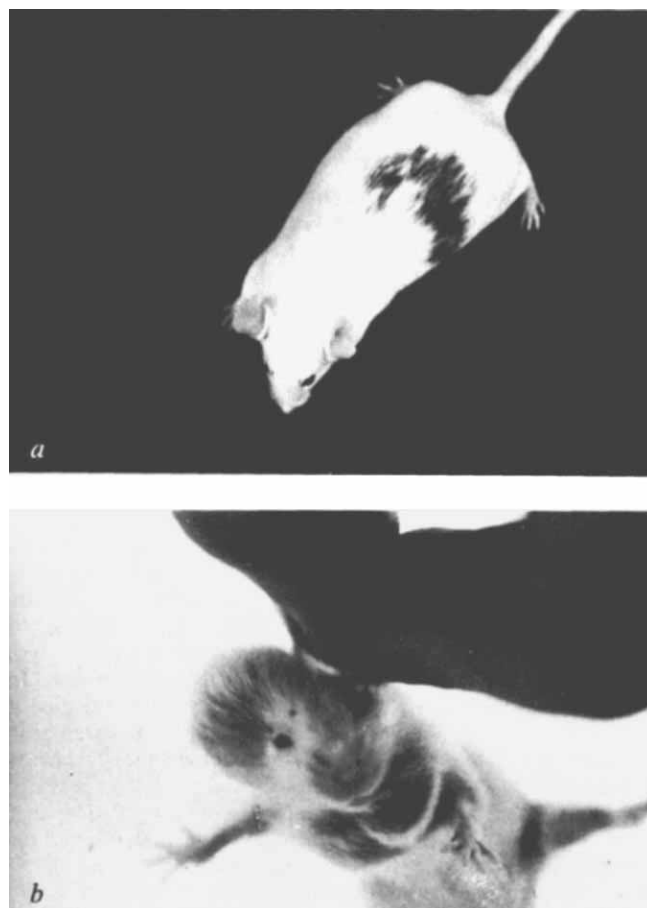


Fig. 1 Chimaeric animals. *a*, Animal T89 at 6 weeks of age showing pigmented area of coat. *b*, Animal T38 at 2 weeks of age showing three subcutaneous tumours in the neck and throat regions.

Table 3 Chimaeric animals

Animal code	Sex	Injected cell line	Animal fate	Age at death (d)	GPI															Tumour location
					Pigment			Brain	Gonads	Gut	Heart	Kidney	Liver	Lung	Skeletal muscle	Spinal cord	Spleen	Thymus	Tumour	
Coat	Eyes	Blood																		
T120	f	SIKR-OSB	k	42	C	?	C	H	C	—	C	C	H	C	C	—	H	C	None	
T84	f	C17	l		H	C	H	—	—	—	—	—	—	—	—	—	—	—	None	
T85	f	C17	l		C	C	H	—	—	—	—	—	—	—	—	—	—	—	None	
T89	f	C17	l		C	C	H	—	—	—	—	—	—	—	—	—	—	—	None	
T6	f	C17	d	7	C	C	C	H	—	C	C	H	H	C	C	H	H	—	C	s.c. under ear
T18	f	C86	k	3	H	H	H	H	—	—	H	H	H	H	C	H	—	—	C	s.c. base of tail
T33	f	C86	k	9	H	H	H	H	—	H	H	H	H	H	C	H	H	H	C	s.c. genital region
T38	f	C86	k	14	H	H	C	H	—	C	C	C	H	C	C	—	H	C	C	cerebellum
T43	m	C86	d	8	H	H	—	H	C	—	H	H	C	—	C	—	C	—	C	s.c. under ear
T65	f	C86	d	26	H	H	H	H	—	—	H	H	H	H	H	—	H	—	C	s.c. throat
T72	m	C86	d	5	H	H	—	H	H	H	H	H	H	C	C	—	—	—	C	s.c. throat
																			C	s.c. neck
																			C	s.c. thorax
																			C	s.c. below eye
																			C	abdominal cavity
																			C	s.c. under ear

k, Killed; d, died; l, live; H, host; C, chimaeric; —, not analysed; ?, not analysable; s.c., subcutaneous.

the trophoblast of a second, and in both portions of a third. Two other conceptuses showed traces of C86-type isozyme. These results indicated that the injected embryonal carcinoma cells had proliferated in some embryos without obviously interfering with their morphogenesis. In subsequent experiments the embryos were allowed to develop to term.

The rate of development of operated blastocysts (Table 2) compares favourably with that found in previous experiments¹⁴ and argues against embryonal carcinoma cells adversely affecting prenatal development in spite of the relatively large number of cells injected into each blastocyst. Of 121 mice born, 11 contained progeny of the embryonal carcinoma cells (Table 3) and 4 of these were normal healthy animals.

Mouse T120, obtained after injection of SIKR-OSB cells, had small patches of agouti hair in the coat, indicating the presence of embryonal carcinoma derived dermal elements interacting in the normal manner in the hair follicles¹⁶. This animal was healthy when killed at 6 weeks of age. Analysis indicated extensive chimaerism of internal tissues.

Mice T6, T84, T85, and T89 were obtained after injection of C17 cells. T6, T85 and T89 exhibited pigmentation in both the coat and eyes whereas T84 showed only eye pigmentation. Three remained apparently healthy at 9 weeks of age and the areas of pigmentation have proved stable in relative size and distribution (Fig. 1a). Apart from blood, GPI analysis for internal chimaerism of these three animals has not yet been undertaken as they are being test bred. The fourth mouse, T6, developed two subcutaneous tumours postnatally and died at 7 d. GPI analysis demonstrated that embryonal carcinoma-derived cells were present in several macroscopically normal tissues in addition to both tumours.

None of the mice obtained after injection of C86 cells exhibited coat or eye pigmentation; however, six developed one or more tumours which were evident at birth or within 6 d. All except one tumour (in T65) grew rapidly postnatally (Fig. 1b), so that all six mice died or were killed during the first 3 weeks of life. All tumours were subcutaneous except one that protruded through the skull from the cerebellar region of the brain and one attached to the liver. The tumours were poorly differentiated

teratocarcinomas, consisting chiefly of embryonal carcinoma; GPI analysis indicated the presence of both embryonal carcinoma-derived and host cells. In addition, five of the six animals showed chimaerism of other macroscopically normal tissues, probably indicating the presence of normal, appropriately differentiated cells derived from the embryonal carcinoma. Because of the tumours, however, we cannot eliminate the possibility of numerous small metastases.

Normal embryonic cells can be converted into teratocarcinomas by transplanting embryos of various developmental stages into ectopic sites in syngeneic adult hosts¹⁷⁻¹⁹. Present experiments suggest that such teratocarcinoma-derived embryonal carcinoma cells taken from culture *in vitro* can participate in normal morphogenesis and differentiation when restored to the environment of the early embryo. We have obtained unequivocal normal chimaeras following the injection of pluripotential cells of two different lines. Embryonal carcinoma progeny in these chimaeras were able to differentiate into apparently normal functional melanocytes of both the coat and iris (T84, T85, T89) and normal dermal elements of hair follicles (T120). In future experiments we intend to use genetic polymorphisms of differentiated functions to investigate whether the embryonal carcinoma progeny in the other chimaeric tissues are functioning like normal differentiated cells of those tissues. Also we are, at present, attempting to increase the level of chimaerism by injecting dissociated cells directly into the inner cell mass of blastocysts to enable more effective interaction between embryonal carcinoma and embryo cells.

All living mice from the present experiments are being test bred to determine whether the embryonal carcinoma cell progeny can colonise the germ line and produce functional gametes. This would raise the possibility of producing mice from a tissue culture cell line selected to carry various mutations and of establishing lines of mice which are models for genetically determined human diseases, or which carry mutant alleles of relevance to studies on development.

We thank Drs S. A. Iles and E. P. Evans for allowing us to enter their data in Table 1; Drs G. R. Martin and C. F. Graham for help and discussions. This work was supported by the

Cancer Research Campaign and the MRC. M.W.McB. was supported by a Canadian MRC Centennial Fellowship.

Note added in proof: Chimaeric mice have been obtained following injection of teratocarcinoma cells from *in vivo* tumours. One chimaera sired 61 offspring, all of which carried genetic markers of the teratocarcinoma cells²¹.

V. E. PAPAIOANNOU
M. W. MCBURNEY
R. L. GARDNER

Department of Zoology,
University of Oxford,
South Parks Road, Oxford, UK

M. J. EVANS

Department of Anatomy and Embryology,
University College London,
Gower Street, London WC2, UK

Received August 6; accepted September 25, 1975.

- ¹ Gardner, R. L., and Papaioannou, V. E., in *The Early Development of Mammals* (edit. by Balls, M., and Wild, A. E.), 107 (Cambridge University Press, Cambridge, 1975).
- ² Gardner, R. L., and Rossant, J., in *Embryogenesis in Mammals*, CIBA Foundation Symposium (edit. by O'Connor, M.), (Elsevier, Amsterdam, in the press).
- ³ Martin, G. R., *Cell*, **5**, 229 (1975).
- ⁴ Damjanov, I., and Solter, D., *Curr. Top. Path.*, **59**, 69 (1974).
- ⁵ Stevens, L. C., *Adv. Morphogen.*, **6**, 1 (1967).
- ⁶ Pierce, G. B., *Curr. Top. dev. Biol.*, **2**, 223 (1967).
- ⁷ Martin, G. R., and Evans, M. J., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1441 (1975).
- ⁸ Nicolas, J. F., Dubois, P., Jakob, H., Guillard, J., and Jacob, F., *Ann. Microbiol.*, **126A**, 3 (1975).
- ⁹ Gardner, R. L., *Nature*, **220**, 596 (1968); Gardner, R. L., *Adv. Biosci.*, **6**, 279 (1971).
- ¹⁰ Gardner, R. L., and Lyon, M., *Nature*, **231**, 385 (1971).
- ¹¹ Ford, C. E., Evans, E. P., and Gardner, R. L., *J. Embryol. exp. Morph.*, **33**, 447 (1975).
- ¹² Brinster, R. L., *J. exp. Med.*, **140**, 1049 (1974).
- ¹³ Staats, J., *Cancer Res.*, **32**, 1609 (1972).
- ¹⁴ Gardner, R. L., *J. Embryol. exp. Morph.*, **28**, 279 (1972).
- ¹⁵ Chapman, V. M., Whitten, W. K., and Ruddle, F. H., *Dev. Biol.*, **26**, 153 (1971).
- ¹⁶ Wolfe, H. G., and Coleman, D. L., in *Biology of the Laboratory Mouse* (edit. by Green, E. L.), (McGraw-Hill, New York, 1966).
- ¹⁷ Stevens, L. C., *Dev. Biol.*, **21**, 364 (1970).
- ¹⁸ Solter, D., Skreb, N., and Damjanov, I., *Nature*, **227**, 503 (1970).
- ¹⁹ Stevens, L. C., *J. Embryol. exp. Morph.*, **20**, 329 (1968).
- ²⁰ Evans, M. J., *J. Embryol. exp. Morph.*, **28**, 163 (1972).
- ²¹ Mintz, B., and Illmensee, K., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Collagen characterisation and cell transformation in human atherosclerosis

COLLAGEN comprises 40–60% of the protein^{1,2} and 30% of the weight³ of human fibrous atherosclerotic plaques. Its deposition in the arterial intima is largely responsible for the occlusive and irreversible nature of coronary and cerebral arterial disease. Four genetically distinct types of collagen have been identified in mammalian tissues and at least five structural genes are involved in their synthesis⁴. The transcription of these various genes seems to be tissue specific. Type I collagen is found in tendon, bone and adult skin, type II is found in cartilage and intervertebral disk, and type IV is restricted to basement membrane. Type III collagen was observed first in newborn human skin⁵ and has been isolated and characterised from human aorta and leiomyoma⁶. Initial reports identified both types I and III in human aorta^{4,7}. We have now found that fibrous atherosclerotic plaques contain proportions of these two collagens which are different from those occurring in normal arterial wall.

Eight aortas each containing large areas of fibrous atherosclerosis were obtained *post mortem* from middle-aged men (mean age 52 yr), washed free of blood and the adventitia stripped and discarded. Raised white intimal plaques were removed and pooled. Residual segments of aortic media were also pooled. The resulting two fractions were treated separately, but identically, in all subsequent procedures.

Minced, defatted tissues were first decalcified by exhaustive stirring with disodium EDTA. Collagen was then extracted from the washed decalcified samples by stirring for 24 h at 4 °C with 50 volumes of 0.5 M acetic acid

containing 200 mg pepsin (Worthington PM, 2,800 U mg⁻¹) per g of lyophilised tissue⁸. Collagen was precipitated from the clarified digest by addition of solid NaCl to a concentration of 0.9 M, redissolved in 0.5 M acetic acid and dialysed exhaustively against 1.0 M NaCl, 0.05 M Tris, pH 7.5. Initial separation of types I and III in each sample was achieved by raising the NaCl concentration in steps of 0.2 M up to 3.0 M. A substantial precipitate of type III collagen was obtained from the aortic medial extract at 1.6 M NaCl, but with atherosclerotic intima the initial precipitate was slight and came down at 1.8 M. At 2.6 M NaCl large amounts of type I collagen were obtained from atherosclerotic intima and smaller amounts from the media.

Samples (20 mg) of each freeze-dried, salt-fractionated precipitate were chromatographed on carboxymethylcellulose (see Fig. 1 for conditions) after denaturation at 45 °C for 40 min. Elution positions of rat skin collagen $\alpha 1(I)$, $\alpha 2$ and $\beta 12$ chains on carboxymethylcellulose are shown in

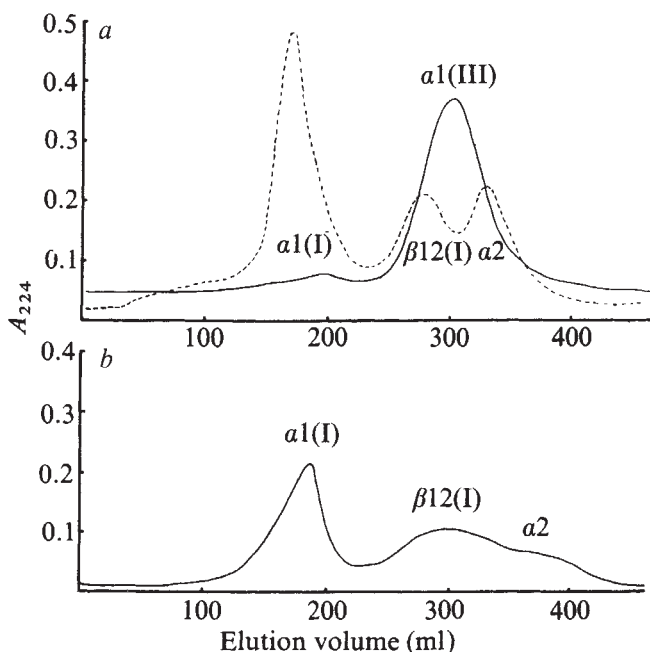


Fig. 1 Carboxymethylcellulose chromatography of aortic collagens on a 16 × 100 mm column at 40 °C in 0.02 M sodium acetate buffer containing 1 M urea, pH 4.8, using a linear gradient of 0.02 M to 0.12 M NaCl over a total volume of 500 ml. *a*, 1.6-M salt fraction from pepsin-treated human aortic media (—) and lathyritic rat skin collagen (---). *b*, 2.6-M salt fraction from pepsin-treated human atherosclerotic intima.

Fig. 1a. The 1.6 M NaCl precipitate from aortic media yielded only a trace amount of $\alpha 1(I)$. Instead, a broad peak eluted in an intermediate position between $\beta 12$ and $\alpha 2$ (Fig. 1a).

The latter component, rechromatographed on an Agarose column previously calibrated with collagen chains and peptides, yielded two major peaks eluting at positions corresponding to V_0 and 270,000 daltons (Fig. 2). Amino acid analysis (given in Table 1) of both major Agarose peaks was identical and seemed very similar to analyses reported for human $\alpha 1(III)$ ⁹. When the 270,000 molecular weight Agarose peak was reduced and alkylated and rechromatographed, a new component was produced with a molecular weight of approximately 95,000 and an amino acid composition corresponding to that of the original peak (Table 1). This suggested that much of the 270,000-dalton material was in the form [$\alpha 1(III)$]₂, held together with interchain disulphide bonds between adjacent cysteine molecules.

Although little collagen was precipitated from medial extracts by 2.6 M NaCl, large amounts were obtained from atherosclerosis intima. The carboxymethylcellulose chrom-

Table 1 Amino acid composition of collagen molecules and chains isolated from human aorta

Amino acid	Media [$\alpha 1(\text{III})$] ₃ Ag ₁	Media $\alpha 1(\text{III})$ Ag ₂	Atherosclerotic intima $\alpha 1(\text{I})$ CMC
3-Hydroxyproline	2	ND	ND
4-Hydroxyproline	127	126	104
Aspartic acid	48	49	41
Threonine	15	15	16
Serine	39	40	34
Glutamic acid	72	73	77
Proline	97	96	118
Glycine	350	367	342
Alanine	99	86	117
Cysteine	2*	1.6†	—
Valine	13	11	17
Methionine	8	7	6
Isoleucine	14	13	7
Leucine	22	22	19
Tyrosine	2	2	1
Phenylalanine	8	9	12
Hydroxylysine	7	7	8
Lysine	28	28	27
Histidine	6	5	3
Arginine	45	46	50

Results are expressed as residues per 1,000 amino acid residues. ND, not determined. Ag₁, 270,000 molecular weight peak from Agarose. V_0 peak had similar composition. Ag₂, 95,000 molecular weight peak obtained from Agarose after reduction, alkylation and rechromatography of Ag₁. CMC, $\alpha 1(\text{I})$ derived from carboxymethyl-cellulose.

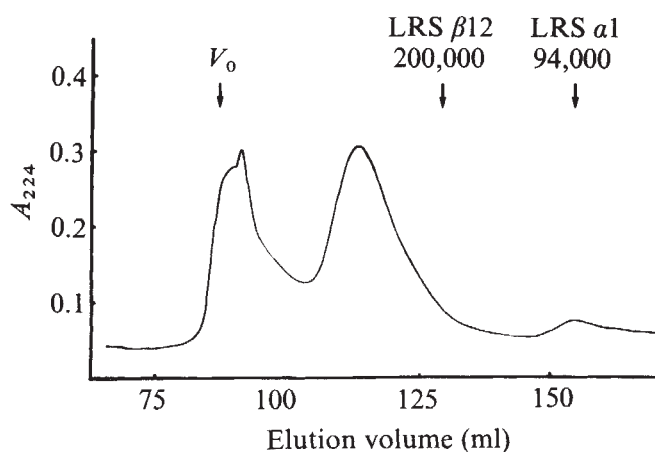
*Determined as cysteic acid after oxidation by performic acid.

†Determined as carboxymethyl cysteine.

atogram of this fraction (type I) is shown in Fig. 1*b*. The initial peak was identified as $\alpha 1(\text{I})$ on the basis of its elution position and amino acid analysis (Table 1). The second broad peak was rechromatographed on Agarose and found to be composed mainly of $\beta 12$ and $\alpha 2$. Thus the 2.6 M salt precipitate from atherosclerotic intima was found to contain almost exclusively components of type I collagen.

From known weights of salt-fractionated precipitates and areas under chromatogram peaks it was calculated that pepsin-extracted aortic medial collagen was approximately 70% [$\alpha 1(\text{III})$]₃ and 30% [$\alpha 1(\text{I})$]₂ $\alpha 2$. In contrast, collagen extracted from atherosclerotic intima contained only 35% [$\alpha 1(\text{III})$]₃, the remaining 65% of the sample being [$\alpha 1(\text{I})$]₂ $\alpha 2$. Because there was some contamination of intimal samples with underlying media, this value for the percentage of type I in atherosclerotic intima is likely to be an under-

Fig. 2 Agarose elution pattern of $\alpha 1(\text{III})$ peak from Fig. 1*a*. Chromatography was performed on a 16 × 1,500-mm column of Bio-Gel A 5 m, equilibrated in 2 M guanidine HCl containing 0.05 M Tris, pH 7.5. Vertical arrows indicate positions of standard collagen chains with their corresponding molecular weights. LRS, lathyritic rat skin.



estimate. The figures also assume that pepsin-solubilised collagen is representative of the whole.

The cells which synthesise these collagens are also different in the two sites. Those of the aortic media are well differentiated smooth muscle cells (SMC) similar to those shown in Fig. 3*a*. Our finding that the collagen of the

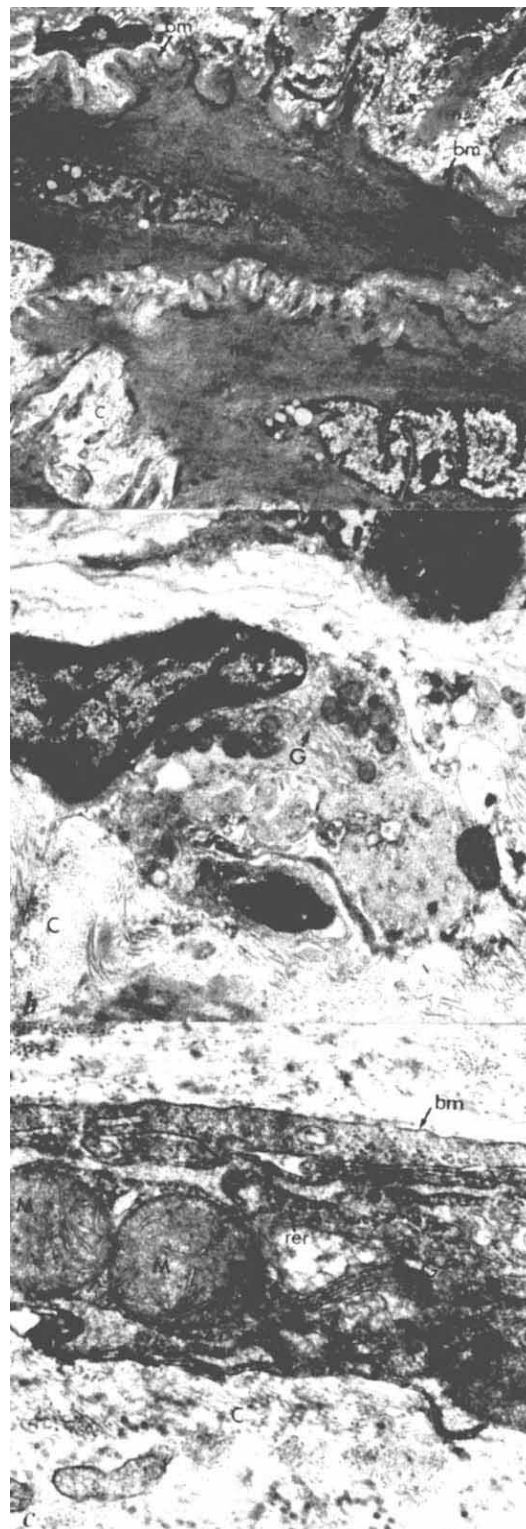


Fig. 3 Electron micrographs of cells from adult human aortae. Stained with uranyl acetate and lead citrate. *a*, Smooth muscle cells from media, × 3,330. *b*, Fibroblasts from atherosclerotic plaque, × 7,030. *c*, Fibroblast from atherosclerotic plaque, × 33,300. N, Nucleus; C, extracellular collagen; M, mitochondria; G, Golgi complex; mf, myofilaments; db, cytoplasmic dense bodies; bm, basement membrane; rer, rough endoplasmic reticulum.

aortic media is mainly type III, together with previous evidence of type III in leiomyomata⁶, suggests that a mixed collagen in which $\alpha 1(\text{III})$ is predominant is a characteristic product of SMC.

It is widely believed that SMC play an important role in atherogenesis^{10,11}. There is considerable experimental evidence that many of the cells of the atherosclerotic plaque are derived from medial SMC which have migrated to the intima and proliferated. These cells often lose some of their distinguishing ultrastructural features and have in consequence been named myointimal cells¹² or 'modified' SMC¹³. If type III collagen could be used as a genetic marker of smooth muscle it would provide a functional criterion on which to base the identity of such cells.

In view of the supposed derivation of plaque cells from SMC the finding of large amounts of type I collagen in the intimal plaques was surprising. The pattern of synthesis is more similar to that of adult skin fibroblasts than it is to smooth muscle. Accordingly, we examined the appearance of the thickened aortic intima in the electron microscope. None of the plaque cells resembled SMC. Myofibrils, basement membrane and cytoplasmic dense bodies were absent. Instead, the cells were smaller and their cytoplasm was packed with ribosomes and organelles (see Fig. 3b and c) and they had the typical appearance of fibroblasts undergoing active protein synthesis¹⁵.

If cells of human fibrous plaques are derived from smooth muscle, it is evident that they have undergone fundamental changes in both morphology and collagen synthesis. Since the new pattern of collagen synthesis would involve change in the rates of translation of genes coding for $\alpha 1(\text{III})$, $\alpha 1(\text{I})$ and $\alpha 2$, we suggest that a basic cell transformation takes place in human atherogenesis. An alternative explanation is that cells of the plaque are derived not from smooth muscle but from fibroblasts resting in normal intima. In that case transformation is unnecessary and it would be best to classify the cells as true fibroblasts. Further study is required to determine which of these interpretations correctly applies to human atherosclerosis.

We thank the Cleveland Clinic, Cleveland, Ohio for autopsy material. The investigation was supported in part by a grant from the US Public Health Service.

KEITH A. MCCULLAGH

Department of Pathology,
University of Bristol, Bristol BS8 1TD, UK

GARY BALIAN

Department of Biochemistry,
University of Washington,
Seattle, Washington 98195

Received July 28; accepted September 19, 1975.

- ¹ Smith, E. B., *J. Atheroscler. Res.*, **5**, 241 (1965).
- ² Anastassiades, T., Anastassiadis, P. A., and Denstedt, O. F., *Biochim. biophys. Acta*, **261**, 418 (1972).
- ³ Levene, C. I., and Poole, J. C. F., *Br. J. exp. Path.*, **43**, 469 (1962).
- ⁴ Miller, E. J., and Matukas, V. J., *Fedn. Proc.*, **33**, 1197 (1974).
- ⁵ Miller, E. J., Epstein, E. H., and Piez, K. A., *Biochem. biophys. Res. Commun.*, **42**, 1024 (1971).
- ⁶ Chung, E., and Miller, E. J., *Science*, **183**, 1200 (1974).
- ⁷ Trelstad, R. L., *Biochem. biophys. Res. Commun.*, **57**, 717 (1974).
- ⁸ Miller, E. J., *Biochemistry*, **11**, 4903 (1972).
- ⁹ Chung, E., Keele, E. M., and Miller, E. J., *Biochemistry*, **13**, 3459 (1974).
- ¹⁰ Wissler, R. W., *J. Atheroscler. Res.*, **8**, 201 (1968).
- ¹¹ Ross, R., and Glomset, J. A., *Science*, **180**, 1332 (1973).
- ¹² French, J. E., *Int. Rev. exp. Path.*, **5**, 253 (1966).
- ¹³ Buck, R. C., in *Atherosclerosis and its Origin* (edit. by Sandler, M., and Bourne, G. H.), 1 (Academic, New York, 1963).
- ¹⁴ Parker, F., and Odland, G. F., *Am. J. Path.*, **48**, 197 (1966).
- ¹⁵ Ross, R., in *Treatise on Collagen*, 2A (edit. by Gould, B.S.) (Academic, New York, 1968).

weight and amino acid composition⁴. These similarities are reflected in methods developed for the isolation of tumour and histocompatibility antigens⁵⁻⁷. Furthermore an inverse relationship has been established between the expression of TAA and H-2 antigens on 3-methylcholanthrene-induced murine sarcomas^{8,9}, whereas the appearance of TAA on aminoazo dye-induced rat hepatomas was associated with deletion of cell surface-expressed normal liver-specific antigens¹⁰.

One interpretation of these findings is that the individually characteristic TAAs on chemically-induced tumours may be modified histocompatibility antigens¹¹. This hypothesis has been examined using a tumour-specific antigen preparation isolated from the serum of Wistar rats bearing transplants of a 4-dimethylaminoazobenzene-induced hepatoma (D23). This antigen, purified from immune complexes in tumour-bearer serum by gel filtration in acidic media followed by affinity chromatography on Sepharose 4B-linked syngeneic rat immune IgG, was homogeneous on isoelectric focusing gels in the presence or absence of neutral detergents. It also reacted specifically with antibody in syngeneic hepatoma D23-immune rat serum as shown by immunodiffusion in isoelectric focused gels and its capacity to neutralise membrane immunofluorescence staining of the serum with hepatoma D23 cells (J.G.B. and R.W.B., unpublished).

The highly purified hepatoma D23 antigen was injected intramuscularly in Freund's complete adjuvant into the hind limb of male New Zealand white rabbits, and after three injections at weekly intervals, the rabbit serum was tested by the indirect membrane immunofluorescence test for the presence of antibody directed against the tumour-specific antigen on viable hepatoma D23 cells (Table 1). The fluorescence index was calculated (FI = (% cells unstained with normal rabbit serum / % unstained with test serum) / % unstained with normal rabbit serum). Fluorescence indices of 0.30 or greater were taken to represent significant staining of hepatoma cells by antibody.

Table 1 Fluorescence indices of rabbit anti-D23 purified serum antigen tested against cells from three Wistar tumours

Serum	Fluorescence index against target cells from:		
	Hepatoma D23	Hepatoma D30	Hepatoma D33
Rabbit anti-D23 purified antigen	1.00, 0.97	1.00, 0.95	0.99, 1.00
Rabbit anti-D23 purified antigen absorbed <i>in vivo</i> *	0.87, 0.79	0.01, 0.10	0.09, 0.07
Rabbit anti-D23 purified antigen absorbed by normal Wistar rat liver membranes†	0.75, 0.83	0.05, 0.03	0.13, 0.12

*Rabbit serum (2 ml) injected intraperitoneally into normal Wistar rats which were bled by cardiac puncture 4 h later.

†Serum (1 ml) absorbed with 50 mg normal Wistar liver membrane protein for 18 h at +4 °C.

The serum from rabbits immunised against the hepatoma D23 purified serum antigen reacted not only against hepatoma D23 cells (FI 1.00–0.97) but also against the two immunologically distinct hepatomas D30 and D33 (FI 0.95–1.00), indicating that this serum recognised antigenic similarities between these three tumours. After absorption *in vivo* or by isolated normal rat liver membranes, the reactivity of the rat antiserum against hepatoma D23 cells was retained (FI 0.75–0.87), whereas that against hepatomas D30 and D33 was lost (FI 0.01–0.13).

Similarly, antisera against the purified hepatoma D23 serum antigen raised in Slonaker rats reacted in a manner similar to the rabbit antiserum (Table 2), so that the unabsorbed antiserum reacted with cell-surface antigens on the three hepatomas used (FI 0.79–0.86). After absorption *in vivo* and *in vitro* with normal Wistar liver membranes, the reactivity against hepatomas D30 and D33 disappeared (FI 0.11–0.20), but that against hepatoma D23 was retained (FI 0.42–0.55).

Tumour-specific antigen related to rat histocompatibility antigens

ATTENTION has been drawn to the similarities between tumour-associated antigens (TAA) and normal histocompatibility antigens, such as their high degree of polymorphism^{1,2}, their cell surface behaviour³, and composition in terms of molecular

Table 2 Fluorescence indices of Slonaker anti-D23 purified serum antigen tested against cells from three Wistar tumours

Serum*	Fluorescence index against target cells from:		
	Hepatoma D23	Hepatoma D30	Hepatoma D33
Slonaker anti-D23 purified antigen	0.83, 0.82	0.79, 0.85	0.86, 0.80
Slonaker anti-D23 purified antigen absorbed <i>in vivo</i>	0.40, 0.42	0.18, 0.20	0.15, 0.17
Slonaker anti-D23 purified antigen absorbed by normal Wistar rat liver membranes	0.45, 0.55	0.17, 0.11	0.19, 0.20

*Absorption conditions as for Table 1.

The antigen used to immunise rabbits and Slonaker rats was highly purified and homogeneous by all the techniques used (J.G.B. and R.W.B., unpublished). Thus, unless the tumour-specific antigen comprised only a small portion of the immunising material and large amounts of histocompatibility antigens were also present, one must suggest that the hepatoma D23 antigen is a modified histocompatibility antigen.

This hypothesis was examined further by labelling the purified hepatoma D23 antigen with ^{125}I (ref. 12) and examining the binding of this labelled antigen to columns of various immunoabsorbents. Four immunoabsorbents were prepared by coupling to CNBr-activated Sepharose respectively, Slonaker antinormal Wistar liver antiserum, normal Slonaker serum, Wistar antihepatoma D30 antiserum and Wistar antihepatoma D23 antiserum. Each gel (5 ml packed volume in PBS) was packed into large Pasteur pipettes containing a plug of glass fibre to prevent loss of gel beads. ^{125}I -labelled D23 antigen (10 ng in 20 μl PBS) was allowed to run on to each column and the sample was washed in to the column with 0.5 ml PBS. Flow was stopped and the columns incubated at room temperature for 3 h, at which time 10 ml PBS were washed through the column at 0.5 ml min $^{-1}$ and fractions collected. Any bound material was eluted with 3-MCF $_3$ COONa pH 7.3 and the eluate dialysed against PBS. Both unbound and eluted material were concentrated to 0.5 ml and tested to see if binding to CNBr-Sepharose-linked Wistar antihepatoma D23 antiserum occurred (Table 3).

Of label added to the Slonaker antinormal Wistar liver immunoabsorbent, 75.4% could be bound and eluted and 81.5% of this could be rebound and eluted from Wistar antihepatoma D23. This parallels the results obtained with an initial column of Wistar antihepatoma D23 in that 77.6% of material could be bound and eluted and 87.5% of this could be

rebound to a similar column. Conversely, with material added to normal Slonaker or Wistar anti-D30 immunoabsorbents, very little was bound and eluted (29.7 and 25.2% respectively) and this material would not rebound to Wistar antihepatoma D23 (17.5 and 0.7%). Material not bound to these columns (65.4 and 71.6% label), however, would bind to the Wistar antihepatoma D23 (89.5 and 85.75% respectively). Thus the purified hepatoma D23 antigen shows points of identity with antigens recognised by Slonaker rats on normal Wistar liver membranes.

In this rat hepatoma D23 system, Baldwin and Graves¹⁰ have shown that when tumour antigens are present there is a deletion of normal antigens recognised by a xenogeneic antiserum. Furthermore, Invernizzi and Parmiani¹¹ have shown that the tumour-associated transplantation antigens of chemically-induced mouse sarcomas crossreacted with allogeneic histocompatibility antigens. They concluded that in this system the tumour antigen was the result of carcinogen-induced mutation at the level of histocompatibility genes, as there seemed to be a linkage between the tumour antigen and the H-2 system. The work reported here confirms and supports the work of Invernizzi and Parmiani¹¹ and shows that the tumour-associated transplantation antigen of a chemically induced tumour is a modified histocompatibility antigen.

J. G. BOWEN*

R. W. BALDWIN

Cancer Research Campaign Laboratories,
University of Nottingham,
University Park,
Nottingham NG1 2RD, UK

Received August 11; accepted September 22, 1975.

*Present address: Laboratory for Experimental Surgery, Erasmus University, Rotterdam, The Netherlands.

- Basombrio, M. A., *Cancer Res.*, **30**, 2458-2462 (1970).
- Graff, R. J., and Bailey, D. W., *Transplant. Rev.*, **15**, 26-49 (1973).
- Yefenof, E., and Klein, G., *Expl Cell Res.*, **88**, 217-224 (1974).
- Baldwin, R. W., Bowen, J. G., and Price, M. R., *Biochim. biophys. Acta*, **367**, 47-58 (1974).
- Baldwin, R. W., and Graves, D., *Clin. exp. Immun.*, **11**, 51-56 (1972).
- Baldwin, R. W., Harris, J. R., and Price, M. R., *Int. J. Cancer*, **11**, 385-397 (1973).
- Harris, J. R., Price, M. R., and Baldwin, R. W., *Biochim. biophys. Acta*, **311**, 600-614 (1973).
- Heywood, G. R., and McKhann, C. F., *J. exp. Med.*, **133**, 1171-1187 (1971).
- Tsakraklides, E., Smith, C., Hersey, J. H., and Good, R. A., *J. natn. Cancer Inst.*, **52**, 1499-1504 (1974).
- Baldwin, R. W., and Graves, D., *Int. J. Cancer*, **9**, 76-85 (1972).
- Invernizzi, G., and Parmiani, G., *Nature*, **254**, 713-714 (1975).
- Greenwood, F. C., Hunter, W. M., and Glover, J. S., *Biochem. J.*, **89**, 114-123 (1963).

Lethality of interferon preparations for newborn mice

WE have previously reported that the daily subcutaneous inoculation of newborn mice with mouse brain interferon preparations was not accompanied by any discernible effect on their growth or development¹. Far more potent interferon preparations have been shown to inhibit cell division in normal adult mice. Thus, the multiplication of allogeneic spleen and syngeneic bone marrow cells was inhibited when injected into X-irradiated adult mice treated for several days with potent interferon preparations², and liver regeneration was inhibited in partially hepatectomised adult mice treated with interferon³. We report here that daily injection of potent interferon preparations results in the death of newborn mice. To our knowledge this is the first report of untoward effects associated with interferon treatment of the normal animal. These results may be relevant to the use of interferon in man.

Newborn Swiss or C3H mice from pathogen-free colonies were injected subcutaneously in the interscapular region with the different preparations. They received daily inoculations of 0.05 ml for 1 week beginning on the day of birth and 0.10 ml daily thereafter for 1 month. In all experiments there were at least two litters of mice per group. In some experiments different treatments were undertaken on

Table 3 Binding of ^{125}I -labelled hepatoma D23 antigen to various immunoabsorbents

Immunoabsorbent	% ^{125}I -labelled hepatoma D23 antigen	
	Unbound	Bound and eluted
Slonaker antinormal Wistar liver	19.5	75.4
Normal Slonaker serum	65.4	29.7
Wistar antihepatoma D30	71.6	25.2
Wistar antihepatoma D23	19.0	77.6
Material unbound on columns of*		
Slonaker antinormal Wistar liver	74.7	20.5
Normal Slonaker	5.4	89.5
Wistar antihepatoma D30	10.8	85.75
Wistar antihepatoma D23	80.0	16.3
Material eluted from column of*		
Slonaker antinormal Wistar liver	15.2	81.5
Normal Slonaker	79.6	17.5
Wistar antihepatoma D30	95.0	0.7
Wistar antihepatoma D23	10.8	87.5

*This material was tested for binding to CNBr-Sepharose-linked Wistar antihepatoma D23.

Table 1 Lethality of mouse interferon preparations for newborn Swiss or C3H mice

Experiment No.	Mouse strain	Type of mouse interferon*	Mouse interferon	Control preparation	Injected with: Inactivated mouse interferon	Human interferon	Uninoculated
1	Swiss	C243	21/21† (13±1)‡	0/23	NT	NT	0/27
2	Swiss	C243	23/23 (11±1)	0/11	NT	NT	0/22
3	Swiss	C243	22/22 (13±2)	NT	NT	NT	0/21
4	Swiss	C243	26/26 (9±1)	0/18	NT	0/23**	0/53
5	Swiss	C243	15/15 (10±1)	NT	0/15	NT	0/19
6	C ₃ H	C243	15/15 (9±1)	0/30	0/15¶	NT	0/18
7	Swiss	L§	6/7 (14±1)	NT	NT	NT	0/18
Total:			128/129	0/82	0/30	0/23	0/178

*A different mouse C243 cell interferon preparation (titre 1:800,000) was used in each of experiments 1–6.

†Number of mice dead/total number of mice injected, based on survival at 1 month. Newborn mice dying in the first 2 d of life are not included.

‡Mean day of death ± s.d.

§Titre of the Sendai virus-induced L cell interferon was 1:240,000.

||Inactivated by heating at 60 °C for 1 h; residual titre 1:100,000.

¶Inactivated by treatment with 0.02 M sodium periodate at pH 4 for 3 h at 4 °C; residual titre 1:600.

**Titre of human leukocyte interferon was 1:2,000,000 (MRC reference interferon (69/19) units) as assayed on human diploid fibroblasts. NT, Not tested.

Mouse interferon from suspension cultures of C243 cells⁴ induced with Newcastle Disease Virus (NDV) was concentrated and semipurified as described previously⁵. Mock interferon was prepared in a manner identical to that used in the preparation of interferon with the exception that the interferon inducer, NDV, was either omitted altogether, or added for 1 h immediately before collecting the culture supernatant (experiment 4). For experiment 7, mouse interferon prepared in monolayer cultures of L cells inoculated with Sendai virus, was concentrated by selective precipitation with ammonium sulphate as described for the C243 cell interferon⁵. All interferon and control preparations were further dialysed for 24 h at 4 °C against 0.01 M perchloric acid before testing for toxicity on a line of L1210 cells resistant to interferon⁶.

Specific activity of mouse C243 cell interferon preparations was in the range $0.7\text{--}3 \times 10^6$ units per mg protein and that of the L cell interferon was 2×10^4 units per mg protein. C243 cell control preparations contained approximately the same amount of protein per ml as the C243 cell interferon (that is, 1.5–3.4 mg ml⁻¹). Partially purified human interferon prepared in leukocyte suspensions inoculated with Sendai virus was generously provided by Dr K. Cantell⁷. Specific activity was 3.5×10^5 units per mg protein. Interferon units quoted are as measured in our laboratory and one of our units equals 4 mouse interferon reference units.

labelled mice within the same litter. All mice were weighed daily. On occasions moribund mice were killed for histological examination of their organs. Some mice in the different groups surviving at 1 month were killed, others were observed for 3–6 months.

Newborn mice inoculated with interferon (titre 1:800,000) seemed to develop and grow normally for the first 6–7 d. In the ensuing few days an increasing difference in weight was observed between interferon-treated mice and their control littermates. Interferon-treated mice died between day 8 and 14. The lethality of mouse interferon preparations for newborn mice is illustrated in Table 1 and the salient points may be summarised as follows: All (122 out of 122) newborn Swiss and C3H mice inoculated daily with C243 cell interferon preparations (titre 1:800,000) died. Six out of seven newborn Swiss mice inoculated with another type of mouse interferon preparation (titre 1:240,000), derived from L cells (and induced with a different virus), also died. No deaths were observed in mice inoculated daily with control preparations (0 out of 82); with inactivated mouse interferon preparations (0 out of 30); with human leukocyte interferon preparations (0 out of 23) or left uninoculated (0 out of 178).

To determine the minimal lethal amount of interferon, newborn Swiss mice were inoculated daily with tenfold dilutions of an interferon preparation (titre 1:800,000). Only mice inoculated with the undiluted interferon preparation died, whereas those inoculated with preparations of titre 1:80,000 or less seemed to grow normally; and no abnormalities were observed on histological examination of their organs at 1 month. These findings are in accord with our previous results¹ indicating that newborn mice inoculated with mouse brain interferon preparations (titre 1:8,000–1:16,000) developed and grew normally without presenting any abnormalities on histological examination. In experiment 5 (Table 1) mouse interferon was only partially inactivated by heating and the residual titre was found to be 1:100,000. Mice treated with these preparations seemed to develop normally. Thus, our results suggest that daily inoculation of newborn mice with interferon preparations of titre 1:100,000–1:240,000 (experiment 7, Table 1) would prove fatal for 50% of the mice.

The most striking abnormality on killing moribund interferon (both C243 and L cell interferon) treated mice was a pale grey liver. Examination of the liver of these mice under the light microscope revealed large areas of cell degeneration and necrosis without any inflammatory reaction (Fig. 1c and d). Examination of liver sections under the electron microscope showed an accumulation of fat, decreased glycogen, swelling of both the mitochondria and the rough endoplasmic reticulum, and discrete nuclear and nucleolar abnormalities. No viral particles were observed. The only other abnormalities on light microscopic examination were: some diminution of the cortical region of the thymus in some mice (and presence of some pycnotic cells in the cortex) and poorly differentiated germinal follicles in the spleens of most mice.

In the experiments described, interferon treatment of newborn mice proved lethal when begun on the day of birth and continued daily. When, however, treatment was initiated on day 6 of life and continued daily for 14 d, mice seemed to grow normally and no mortality was observed. Two mice were killed on day 21 and histological examination of their livers revealed an increased granularity of the cytoplasm without cellular degeneration. The resistance of these mice may be related to the stage of differentiation of the liver at the time interferon inoculations were begun.

We have no reason to believe that the interferon preparations exerted a nonspecific toxic effect, or that an infectious agent was responsible for the hepatic lesions observed in interferon-treated mice. The results of pertinent experiments may be summarised as follows: First, all interferon and control preparations were incubated (1:10 final dilution, that is, 80,000 interferon units) with interferon-resistant L1210 cells⁶. Neither the multiplication nor the viability of these cells was affected over a 48-h period. Representative interferon preparations exerted no detectable toxic effect on confluent monolayer cultures of mouse L or chick embryo cells. Second, one litter of 10 newborn Swiss mice was inoculated intracerebrally daily for 3 d with a C243 cell interferon (titre 1:800,000). All mice survived. No toxic effects were noted and no abnormalities were observed over a 3-month period. Third, two moribund interferon-treated mice with gross liver abnormalities (confirmed

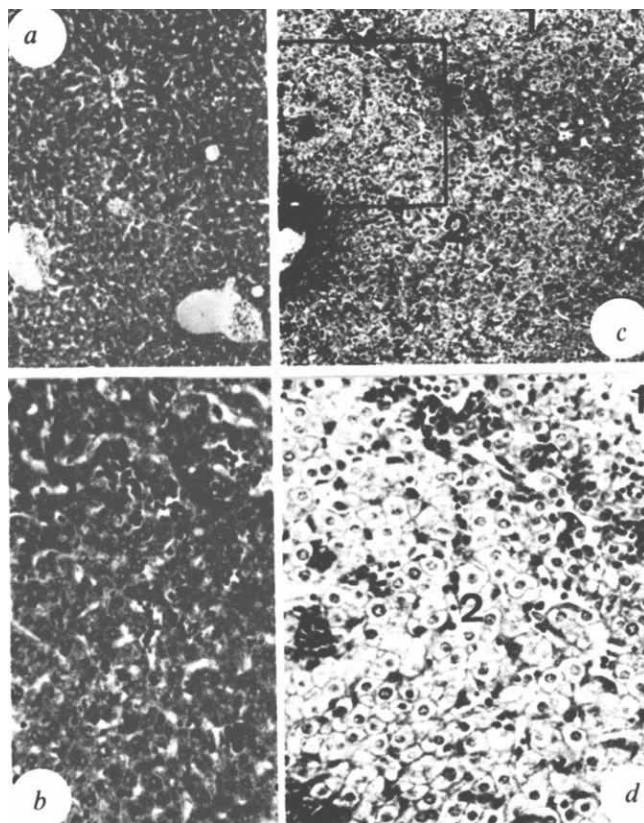


Fig. 1 Sections of liver of 10-d-old Swiss mice. *a* and *b*, Untreated ($\times 75.6$ and $\times 189$); *c* and *d*, treated with a C243 interferon preparation (titre 1:800,000; $\times 75.6$ and $\times 189$) showing focal area of necrosis (1) and diffuse cellular degeneration (2).

on light and electron microscopic examination) were killed and extracts of liver and kidney were inoculated subcutaneously into newborn mice and into primary cultures of newborn mouse kidney. No abnormalities were observed in newborn mice kept for 3 months. No cytopathic agent was detected in cell culture. Using immunofluorescent techniques, inoculated cell cultures did not seem to contain either lymphocytic choriomeningitis or murine hepatitis viral antigens.

Our results show therefore that daily inoculation of newborn mice with potent interferon preparations is lethal and that mice die with extensive liver damage. Our findings may be relevant to the treatment of patients with exogenous interferon. If we suppose that a newborn baby is as sensitive to human interferon preparations as a newborn mouse is to mouse interferon, then our results suggest that the daily inoculation of 50 ml human interferon preparation (titre 1:800,000) might represent a lethal dose for a 3-kg baby (that is, compared with 0.05 ml for a 3-g mouse). Our results also raise the possibility that lower doses of interferon may cause adverse effects in newborn infants that are not immediately apparent. We are aware of the hazard of this analogy, but patients are currently being treated with potent interferon preparations and it is therefore incumbent on clinicians to be aware of the potential dangers of interferon therapy at least in infants. On the other hand it is also important to emphasise that adult patients have been treated twice or three times weekly for extended periods of time with potent leukocyte interferon preparations without showing any untoward effects^{8,9}.

We thank Ms J. Begon-Lours, J. Buywid, and M. T. Bandu for assistance; Dr C. Huet and Professor W. Bernhard for the electron microscopic examination of liver; and Dr J. C. Guillon for his attempts to isolate infectious agents from the liver. M.G.T. thanks the Philippe Foundation.

This work was supported in part by INSERM, CNRS and the Fondation pour la Recherche Médicale.

ION GRESSER
MICHAEL G. TOVEY
CHANTAL MAURY
IVAN CHOUROULINKOV

*Institut de Recherches Scientifiques sur le Cancer,
BP 8, 94800 Villejuif, France*

Received July 24; accepted September 25, 1975.

- ¹ Gresser, I., and Bourali, C., *Eur. J. Cancer*, **6**, 553-556 (1970).
- ² Cerottini, J. C., Brunner, K. T., Lindahl, P., and Gresser, I., *Nature new Biol.*, **242**, 152-153 (1973).
- ³ Frayssinet, C., Gresser, I., Tovey, M. G., and Lindahl, P., *Nature*, **245**, 146-147 (1973).
- ⁴ Oie, H. K., Gazdar, A. F., Buckler, C. E., and Baron, S., *J. gen. Virol.*, **17**, 107-109 (1972).
- ⁵ Tovey, M. G., Begon-Lours, J., and Gresser, I., *Proc. Soc. exp. Biol. Med.*, **146**, 809-815 (1974).
- ⁶ Gresser, I., Bandu, M. T., and Brouty-Boyd, D., *J. natn. Cancer Inst.*, **52**, 553-559 (1974).
- ⁷ Cantell, K., Hirvonen, S., Mogensen, K. E., and Pyhälä, L., *In vitro Monograph* No. 3 (edit. by Waymouth, C.) (Rockville, Maryland, 1973).
- ⁸ Strander, H., Cantell, K., Carlström, G., and Jakobsson, P. A., *J. natn. Cancer Inst.*, **51**, 733-742 (1973).
- ⁹ Ahström, L., Dohlwitz, A., Strander, H., Carlström, G., and Cantell, K., *Lancet*, **ii**, 166-167 (1974).

Effect of cortisol, prolactin and ADH on the amniotic membrane

VERY little is known about the endocrineregulation of amniotic fluid volume and composition^{1,2}. Vizsolyi and Perks² have described a technique for studying the transfer of fluid across the amniotic membrane of the guinea pig *in vitro*. They showed that arginine vasotocin and arginine vasopressin (antidiuretic hormone, ADH) could stimulate the movement of fluid from the maternal to the foetal side of the membrane. Because prolactin is found in remarkably high concentrations (up to 10 $\mu\text{g ml}^{-1}$) in primate amniotic fluid^{3,4} and affects renal function⁵⁻⁸, we have investigated the effect of prolactin as well as of arginine vasopressin on the guinea pig amniotic membrane. Because cortisol can reverse the usual antidiuretic effect of ADH and because prolactin can restore this effect⁹, we have also investigated the action of cortisol. We have shown that ADH and prolactin have opposite effects on the amniotic membrane and that, in both cases, their actions can be reversed by cortisol. We suggest that fluid transfer across the amniotic membrane may provide an insight to the functioning of the kidneys, the ciliary body and the choroid plexus.

The apparatus used was similar to that described by Vizsolyi and Perks². Female guinea pigs were killed in the last three weeks of pregnancy. Two portions of the amniotic membrane were tied over the ends of two glass tubes, each with an end area of 1 cm²; the foetal surfaces of the membrane faced into the tubes. The tubes were filled to a depth of 10 cm with 'amniotic' saline (Fig. 1) and suspended in oxygenated baths of 'maternal' saline with the fluid level in the tubes 2 cm above that in the bath. The tubes did not float, but were supported by balanced levers connected to rods passing through Sangamo Weston displacement transducers. Changes in weight of the tubes resulting from fluid movements produced movements of the levers and changes in transducer output which were continuously recorded using Devices equipment. The system was calibrated by adding known volumes of fluid to the tubes. Both the relative osmolarities and the 2-cm H₂O pressure head favoured movements of fluid from the tube ('foetal' side) to the bath ('maternal' side). Eight animals were used in preliminary experiments in which various hormone concentrations were tested. Two sets of six experiments were then carried out as indicated in Table 1; in each experiment, the *A* and *B* membranes came from the same animal. The results are shown in Figs 1 and 2. All hormones were added to the foetal side of the membrane.

In the control period no significant movement of fluid

Table 1 Experimental addition of hormones to the foetal side of the amniotic membrane *in vitro*

Experiment	Periods*			
	1	2	3	4
1A	Control	Prolactin (200 ng ml ⁻¹)	Prolactin (1,000 ng ml ⁻¹)	Prolactin (1,000 ng ml ⁻¹) + cortisol (200 ng ml ⁻¹)
1B	Control	ADH (0.05 IU ml ⁻¹)	ADH (0.1 IU ml ⁻¹)	ADH (0.1 IU ml ⁻¹) + cortisol (200 ng ml ⁻¹)
2A	Control	Cortisol (200 ng ml ⁻¹)	Cortisol + ADH (0.05 IU ml ⁻¹)	
2B	Control	Cortisol (200 ng ml ⁻¹)	Cortisol + prolactin (1,000 ng ml ⁻¹)	

* Each period lasted for 30 min.

occurred in either direction. Prolactin at 200 ng ml⁻¹ caused a transfer of fluid to the maternal side; 1,000 ng ml⁻¹ caused a further movement in the same direction. The addition of cortisol (200 ng ml⁻¹) to the prolactin completely reversed the direction of fluid movement. The opposite was found with ADH (0.05 and 0.1 IU ml⁻¹); it caused a movement to the foetal side which was reversed by cortisol. In the second series of experiments, cortisol added alone caused a fluid movement to the maternal side. ADH then caused a further movement in the same direction but prolactin had the reverse effect, moving fluid to the foetal side.

With respect to the amniotic fluid, the prolactin was used in concentrations which are, in primates at least, physiological⁸ whereas the other two hormones were probably at concentrations much greater than those found in amniotic fluid^{9,10}. We therefore suggest that the effect of prolactin alone is likely to be the one which is of practical importance. If prolactin does cause a movement of fluid out of the amniotic sac this could account for the hydramnios which is frequently found when human amniotic fluid prolactin concentrations are low³.

It is already known that prolactin, cortisol and ADH can

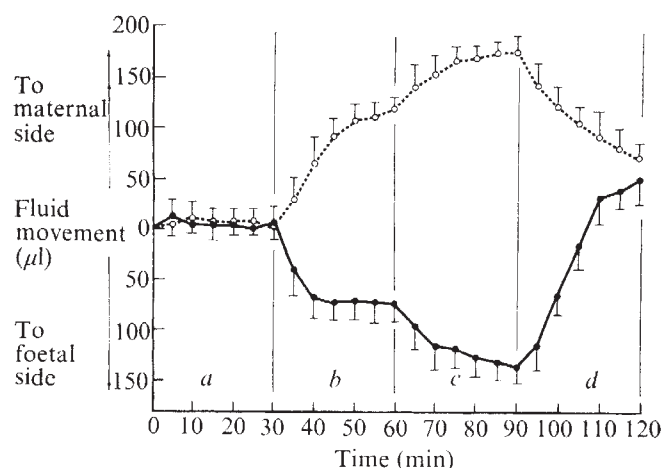


Fig. 1 The fluid movements during the various incubation periods in experiments 1A and 1B (Table 1). At each point the net amount of fluid which has moved since time zero is indicated. Each figure represents the mean of six experiments and the bars indicate the s.e.m. The 'amniotic' saline had the following composition: sodium 125.0, potassium 6.2, calcium 4.5 and magnesium 2.3 mM l⁻¹, all added as chloride; 1.0 g l⁻¹ glucose; 10 ml phosphate buffer at pH 7.8 was added per litre, giving a final pH of 7.4 and osmolality of 286 mosmol l⁻¹. The 'maternal' saline was made up as follows: sodium 150.0, potassium 5.5, calcium 4.4, magnesium 2.6 mM l⁻¹, all added as for the amniotic saline giving a final pH of 7.4 and osmolality of 314 mosmol l⁻¹. The maternal saline was bubbled continuously with oxygen and maintained at 37 °C. Vizsolyi and Perks² studied the compositions of guinea pig amniotic fluid and maternal plasma and devised the 'amniotic' and 'maternal' salines to reproduce the natural fluids as closely as possible. a, Control; b, prolactin (200 ng ml⁻¹), ADH, 0.05 IU ml⁻¹; c, prolactin (1,000 ng ml⁻¹), ADH, (0.1 IU ml⁻¹); d, prolactin (1,000 ng ml⁻¹) plus cortisol (200 ng ml⁻¹), ADH, (0.1 IU ml⁻¹) plus cortisol (200 ng ml⁻¹). ○, Prolactin; ●, ADH.

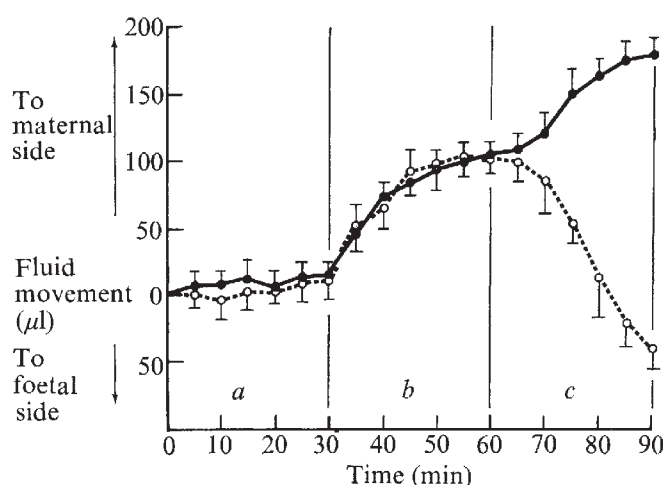


Fig. 2 The fluid movements during the various incubation periods in experiments 2A and 2B. a, Control; b, cortisol (200 ng ml⁻¹); c, cortisol (200 ng ml⁻¹) plus ADH (0.05 IU ml⁻¹) or prolactin (1,000 ng ml⁻¹). Each point represents the mean of six experiments and the bars indicate s.e.m. ●, ADH; ○, Prolactin.

interact in the formation of urine⁷⁻⁹ and of aqueous humour¹¹. Cortisol and prolactin have been shown to have opposite effects on some fish bladders^{12,13}. Because of its relative simplicity, the guinea pig amniotic membrane may provide a good mammalian model for our understanding of amniotic fluid regulation and as an indication as to what may be happening at other sites where urine, aqueous humour, cerebrospinal fluid and gastrointestinal secretions are formed.

We thank the Leverhulme Trust, the World Health Organization, the Ernest Hart Fund of the British Medical Association and Hoechst for financial support.

M. S. MANKU*
J. P. MTABAJI
D. F. HORROBIN*

Department of Physiology,
Newcastle University Medical School,
Newcastle upon Tyne NE1 7RU, UK

Received July 28; accepted September 26, 1975.

*Present address: Clinical Research Institute, 110 Pine Avenue West, Montreal, Canada.

- Liley, A. W., in *Pathophysiology of Gestation*, II (edit. by Assali, N. S., and Brinkman, C. R.), 154-182 (Academic, New York and London, 1972).
- Vizsolyi, E., and Perks, A. M., *Can. J. Zool.*, **52**, 371-386 (1974).
- Friesen, H., et al., in *Prolactin and Carcinogenesis* (edit. by Boyns, A. R., and Griffiths, K.), 64-80 (Alpha Omega Alpha Publishing Co., Cardiff, 1972).
- Josimovich, J. B., Weiss, G., and Hutchinson, D. L. *Endocrinology*, **94**, 1364-1371 (1974).
- Lockett, M. F., *J. Physiol., Lond.*, **181**, 192-199 (1965).
- Horrobin, D. F., et al., *Lancet*, **ii**, 352-354 (1971).
- Horrobin, D. F., *Prolactin: Physiology and Clinical Significance* (Medical and Technical Publishing, Lancaster, 1973).
- Horrobin, D. F., *Prolactin 1974*. (Medical and Technical Publishing, Lancaster, 1974).
- Horrobin, D. F., Manku, M. S., and Robertshaw, D., *J. Endocr.*, **58**, 135-136 (1973).

¹⁰ Cope, C. L., *Adrenal Steroids and Disease*, Second ed. (Pitman Medical, London, 1972).

¹¹ Niederer, W., Richardson, B. P., and Donatsch, P., *Expl Eye Res.*, **20**, 329–340 (1975).

¹² Johnson, D. W., *Am. Zool.*, **13**, 799–818 (1973).

¹³ Doneen, B. A., and Bern, H. A., *J. exp. Zool.*, **187**, 173–179 (1974).

Action of progesterone on preoptic thermosensitive neurones

FEMALE body temperature rises by about 0.5 °C at or near ovulation and remains elevated during the luteal phase of the menstrual cycle. Sustained elevation of basal body temperature is observed also during early pregnancy. These effects have been attributed to progesterone, but little is known about the mechanisms involved¹. We have now found that the hormone affects the activity of the thermosensitive neurones in the preoptic area of the brain.

These neurones change their discharge frequency in response to changes in local brain temperature: that of warm-sensitive neurones increases with a rise in temperature^{2–3}, while that of cold-sensitive neurones increases with a fall in temperature⁴. Intravenous pyrogen in anaesthetised animals (cat and rabbit) decreases the activity of warm-sensitive neurones and increases that of cold-sensitive neurones^{5–8}.

Our investigation followed preliminary studies of 23 unanaesthetised female rabbits, injected intravenously with progesterone (more than 5 mg kg⁻¹). There was a rise in rectal temperature of 0.6 °C, with the highest elevation in core temperature 2 h after injection. Progesterone was dissolved in propylene glycol, but the solvent alone did not cause temperature to rise. We have therefore investigated the effect of systemic administration of progesterone on the thermosensitive neurones.

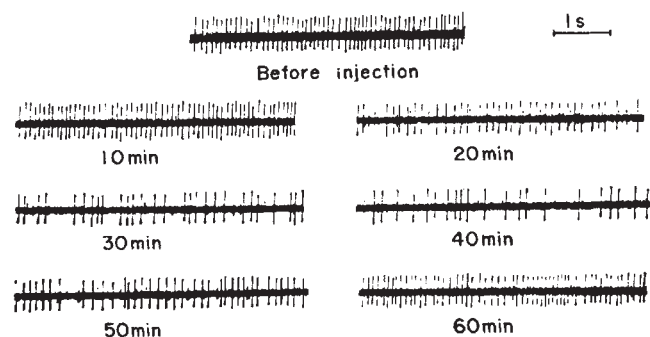


Fig. 1 Discharges of the preoptic warm-sensitive neurone in response to intravenous administration of progesterone in the female rabbit.

Thirty-four female rabbits (2.5–4.0 kg) were anaesthetised with urethane (1 g kg⁻¹), tracheotomised and mounted on a stereotaxic apparatus. (Details of the method have been described before³.) A thermode was implanted in the preoptic area, 1.5 mm lateral to the midline, and brain temperature was measured by a thermocouple placed in the frontal plane, 3.0 mm from the thermode. Impulses of single neurones were recorded on tape and impulses per second were counted with a data processing computer. A small amount of iron was then deposited electrolytically at the tip of the electrode for histological localisation. A neurone with a regular rate of firing was identified as warm-sensitive, cold-sensitive or temperature-insensitive by its responses to local warming and cooling. The effect of progesterone was observed on twenty warm-sensitive, seven cold-sensitive and five insensitive neurones. Progesterone was dissolved in 50% propylene glycol (5 mg ml⁻¹). When the thermal response of a neurone had been observed, the brain temperature was kept at 37.5 °C and progesterone (10 mg in 2 ml) was injected intravenously. In a typical warm-sensitive neurone (Fig. 1), the average rate of firing before injection

was 11.2 impulses s⁻¹. After a latency of about 20 min, this decreased to 7.7 at 24 min, to 6.6 at 40 min and returned to 10.0 at 60 min. Of the 20 warm-sensitive neurones tested, 15 decreased their firing rate by 38–70% after administration of progesterone. The thermal responses of these 15 neurones had not changed when observed 60 min after administration, with the exception of one neurone which showed a decreased thermosensitivity. The rate of firing of five warm-sensitive neurones was unchanged for 2 h after intramuscular administration of progesterone.

In seven cold-sensitive neurones the rate of firing increased after administration of progesterone given intramuscularly in three cases and intravenously in the other two. In one of these neurones, the rate of firing increased from 6.4 to 12.0 impulses s⁻¹ 6 min after injection. Five thermally-insensitive neurones were not affected by progesterone and two warm-sensitive neurones were not affected by propylene glycol.

Plasma concentration of progesterone rises quickly after intravenous administration and returns to a control level in about 1 h (refs 9 and 10). This may explain the short duration of action of progesterone that we observed. We do not know whether the thermosensitive neurones tested in this study are true thermoreceptors or interneurons transmitting temperature signals in a complex neural network of temperature regulation. Nor are we certain whether the neuronal effects which we observed were caused by progesterone *per se* or by its metabolites. Furthermore, the quantitative relationship between oestrogen and progesterone in the luteal phase is complex and oestrogenic hormones are known to cause a slight decrease in body temperature^{11–12}.

It seems likely, however, that the decreased activity of warm-sensitive neurones and the increased activity of cold-sensitive neurones in the preoptic area result in an upward shift of the 'set point' of body temperature, as with the effects of pyrogen. The rise of basal body temperature in the luteal phase seems to be related, at least in part, to the direct or indirect action of progesterone on preoptic thermosensitive neurones.

TERUO NAKAYAMA
MASATOSHI SUZUKI

Department of Physiology,
Osaka University, School of Medicine,
Kita-ku, Osaka 530

NAOTAKA ISHIZUKA

Department of Gynecology and Obstetrics,
Nagoya University, School of Medicine,
Showa-ku, Nagoya 466 Japan

Received August 4; accepted September 12, 1975.

¹ Kappas, A., and Palmer, R. H., *Pharmac. Rev.*, **15**, 123–167 (1963).

² Nakayama, T., Eisenman, J. S., and Hardy, J. D., *Science*, **134**, 560–561 (1961).

³ Nakayama, T., Hammel, H. T., Hardy, J. D., and Eisenman, J. S., *Am. J. Physiol.*, **204**, 1122–1126 (1963).

⁴ Hardy, J. D., Hellon, R. F., and Sutherland, K., *J. Physiol., Lond.*, **175**, 242–253 (1964).

⁵ Cabanac, M., Stolwijk, J. A. J., and Hardy, J. D., *J. appl. Physiol.*, **24**, 645–652 (1968).

⁶ Wit, A., and Wang, S. C., *Am. J. Physiol.*, **215**, 1160–1169 (1968).

⁷ Eisenman, J. S., *Am. J. Physiol.*, **216**, 330–334 (1969).

⁸ Nakayama, T., and Hori, T., *J. appl. Physiol.*, **34**, 351–355 (1973).

⁹ Zarrow, M. X., Shoger, R. L., and Lazo-Wasem, E. A., *J. clin. Endocr. Metab.*, **14**, 645–652 (1954).

¹⁰ Ishizuka, N., *J. Jap. Obstet. Gynec. Soc.*, **9**, 907–925 (1957).

¹¹ Buxton, C. L., and Atkinson, W. B., *J. clin. Endocr. Metab.*, **8**, 544–549 (1948).

¹² Israel, S. L., and Schneller, O., *Fert. Steril.*, **1**, 53–64 (1950).

Excitation of phasically firing supraoptic neurones during vasopressin release

THE neurohypophyseal hormones vasopressin and oxytocin are synthesised within separate neurones of the hypothalamic supraoptic and paraventricular nuclei¹ and each hormone can be liberated independently. Haemorrhage² and bilateral

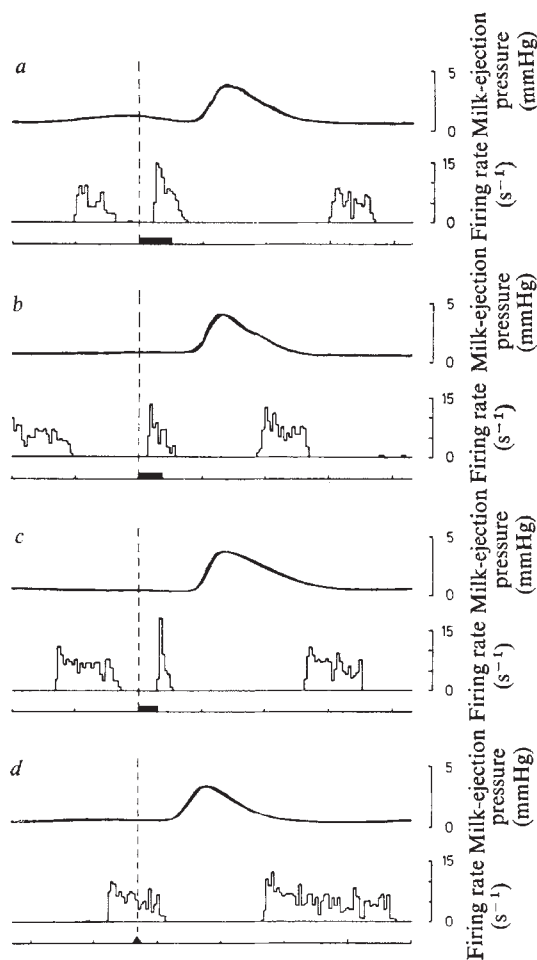


Fig. 1 Responses of a phasically active supraoptic neurone and of intramammary pressure to bilateral carotid occlusion. Antidromically identified neurone fired with bursts of activity separated by silent intervals. Bilateral occlusion of the common carotid arteries (black bars) for 15 s (*a*), 11 s (*b*) and 9 s (*c*) evoked a short supplementary burst of activity from the neurone 4–9 s after the onset of occlusion (dashed line). In two instances (*a* and *c*) the peak firing of the evoked burst exceeded that of the spontaneously occurring bursts. The rise in intramammary pressure occurred 18–20 s after the onset of each occlusion, and was of similar amplitude and duration to that occurring 16 s after the intravenous injection of 250 μ U arginine vasopressin in the black triangle in (*d*). Time base, 30 s.

carotid occlusion³ release vasopressin without oxytocin, whereas oxytocin is released without vasopressin during suckling^{4,5} and parturition⁶. The neuroendocrine cells produce all-or-none action potentials which propagate to the neural lobe, where they trigger hormone secretion⁷. Action potential firing of individual endocrine cells falls into two principal categories: most fire continuously and randomly at 1–2 spikes s^{-1} , while the rest have a phasic pattern of firing, periods of bursting activity alternating every 10–60 s with periods of silence⁸.

Lincoln and Wakerley⁹ recorded the firing rates of supraoptic neurones in rats during suckling, and found that the reflex excited predominantly the randomly firing neurones. We have therefore investigated supraoptic neuronal activity during stimulation which is known to release only vasopressin. We found that, during bilateral occlusion of the common carotid arteries, supraoptic neurones are excited and that this activation is confined almost exclusively to phasically active neurones.

We used lactating female rats under urethane anaesthesia (1.2 mg kg^{-1}). Their common carotid arteries were occluded by a clamp which exerted no tension. Blood pressure and intramammary pressure were measured routinely. The brain was exposed from the ventral surface and supraoptic neurones

were identified by antidromic invasion after electrical stimulation of the pituitary stalk¹⁰.

Of 101 cells studied, 60 had a random pattern of firing, 33 had a phasic pattern and 8 were silent. Bilateral occlusion of the common carotid arteries reproducibly excited 30 of the phasically firing neurones. Occlusion of the arteries during the silent period of a cycle was followed by a burst of action potentials which started less than 10 s after the onset of occlusion. If applied during a spontaneous burst, occlusion often prolonged that burst. Random neurones responded to occlusion in a much more varied manner: 4 were excited, 14 showed slight excitation followed by prolonged inhibition, 24 showed inhibition alone and 18 were unaffected. Of the silent cells, 4 were excited.

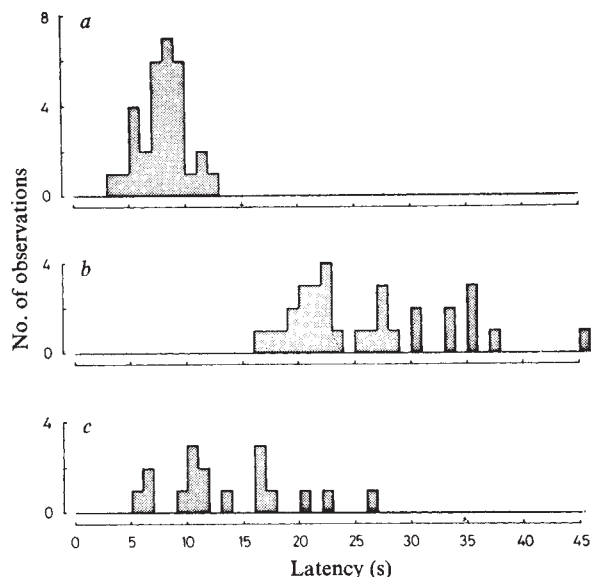
The response of a phasic neurone in a lactating rat to carotid occlusions of various duration is illustrated in Fig. 1. The neurone responded to periods of occlusion of 15, 11 and 9 s with a burst of activity starting 4–9 s after the onset of occlusion.

In Fig. 1*a* and *c* the evoked bursts had a higher peak rate than the spontaneous bursts. Approximately 20 s after the onset of neuronal firing, intramammary pressure increased, showing that one or both neurohypophysial hormones had been released. The increase in pressure was approximately equivalent to that which followed the injection of 250 μ U arginine vasopressin into a jugular vein. In this neurone the duration of the evoked burst seemed to be controlled by the duration of the occlusion; this was also seen in some other neurones.

Recording of intramammary pressure in lactating rats takes advantage of the fact that vasopressin causes milk ejection even though its activity is only about 20% of that shown by oxytocin. If the milk-ejection response to carotid occlusion was caused by hormone released as a result of activation of supraoptic neurones, the response should begin approximately one circulation time after the onset of the evoked burst of activity. This occurred regularly, as shown in Fig. 2.

The mean latency of the milk-ejection response was 26.2 s. This must be explained by the delay before the cells are activated and the time taken by the hormone to reach the mammary

Fig. 2 Histogram of the latencies of neuronal and milk-ejection response to bilateral carotid occlusion and to exogenous vasopressin. Results from eight supraoptic neurones. *a*, Latencies between the beginning of occlusion and the onset of the stimulus-evoked burst of action potentials ranged between 3 and 13 s, with mean latency ($\pm$ s.e.) of 8.1 ± 0.4 s ($n = 31$). *b*, Latencies between the beginning of the same occlusions as in *a* and the onset of the stimulus-evoked milk-ejection response. Mean latency ($\pm$ s.e.) was 26.2 ± 1.3 s ($n = 31$). *c*, Latencies between the injection into the jugular vein of 0.25–1.0 mU vasopressin and the onset of the ensuing milk-ejection responses. Mean ($\pm$ s.e.), 13.7 ± 1.4 s ($n = 17$).



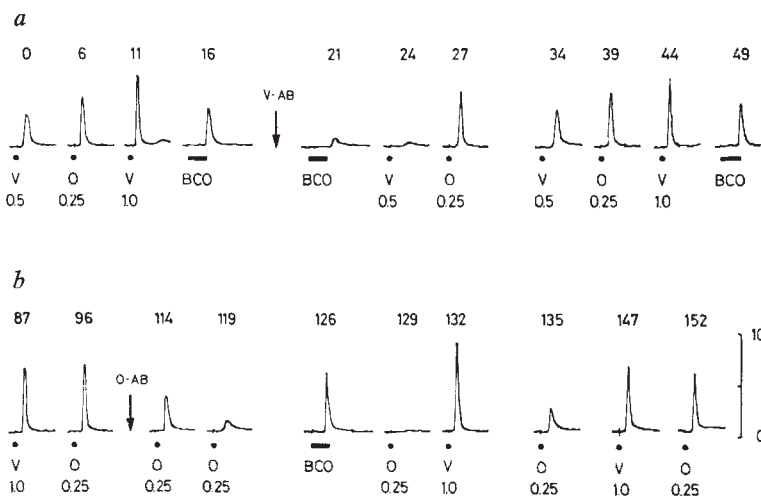


Fig. 3 Effects of injected antibodies to vasopressin and oxytocin on milk-ejection responses to bilateral carotid occlusion and to exogenous neurohormones. Records show milk-ejection responses after exogenous vasopressin (V), exogenous oxytocin (O) and 30 s bilateral carotid occlusion (BCO) before and after intravenous injection into the jugular vein of antibodies at the arrows. Numbers beneath records give concentrations of hormones in mU. Vertical scale is in mmHg. Figures above the responses indicate time (min) after the start of the experiment. Injections or occlusions were made at approximately 5-min intervals, except immediately after antibody injection. The responses are consecutive, but the record is not continuous. *a*, Effect of vasopressin antibodies (V-AB). BCO caused milk-ejection responses equivalent to 0.5 mU vasopressin and slightly less than 0.25 mU oxytocin. After injection of antibodies, responses to BCO and vasopressin are almost abolished whereas response to oxytocin is unaffected. *b*, Effect of oxytocin antibodies (O-AB). Injection of antibodies at arrow progressively abolishes response to 0.25 mU oxytocin without reducing responses to either BCO or vasopressin. Note reversibility of the effects of both antibodies.

gland after release. The observed latency of the onset of the cell response was 8.1 s, and the observed latency of milk ejection following injection of vasopressin into a jugular vein was 13.7 s. The difference between the observed latency of the milk-ejection response to occlusion and the sum of the latter two figures is because the cells do not reach their peak firing rate immediately and because injections into the jugular vein underestimate the time taken by hormone to reach the mammary gland from the pituitary.

There is evidence that carotid occlusion liberates only vasopressin in cat and dog¹¹, but there is no published evidence that this is so in rats. Since both neurohypophysial hormones increase intramammary pressure, it was necessary to establish that the responses we observed were due to vasopressin and not to oxytocin. We used two approaches. First, rats anaesthetised by methane were subjected to a 60-s occlusion and then decapitated. The extracted trunk plasma¹² was assayed by radioimmunoassay for vasopressin and oxytocin¹³ and compared with that of extracts from rats subjected to the same surgery but omitting carotid occlusion. The results showed that occlusion increased mean ($\pm$ s.e.) plasma vasopressin from 303 ± 70 to 970 ± 199 pg ml⁻¹ ($n = 8$; t test, $P < 0.01$) but the increase in oxytocin was much smaller and not significant ($P > 0.1$). Second, intrajugular injections of the anti-vasopressin and the anti-oxytocin antibodies were given to lactating rats in conjunction with bilateral carotid occlusion. Results from one animal are shown in Fig. 3. Injection of 0.2 ml of undiluted rabbit plasma containing the specific anti-vasopressin antibody¹³ depressed the milk-ejection responses to carotid occlusion and to intravenously injected arginine-vasopressin, without affecting that to exogenous oxytocin. Conversely the oxytocin antibody abolished the response to oxytocin but to neither occlusion nor vasopressin.

Occlusions of 5–10 min duration cause vasopressin secretion in cats and dogs, and following section of the sinus nerves, hormone secretion is abolished, indicating that the response is mediated neurally¹¹. Our results indicate that in the rat occlusion of much shorter duration is a stimulus for vasopressin secretion and excites nearly all phasically firing supraoptic neurones; most randomly firing cells are either inhibited or unaffected.

These observations complement the report that, during naturally occurring milk ejection in rats, about half of the randomly firing supraoptic neurones were powerfully excited, whereas phasic neurones were not involved⁹. These findings suggested that randomly firing neurones are those that secrete oxytocin. Since the excitatory input to the supraoptic nuclei in response to bilateral carotid occlusion is apparently channelled almost exclusively to the phasically active neurones, these

cells might represent a distinctive class of vasopressin-secreting neurones.

We thank the Swiss National Science Foundation for support. M. C. H. is an IBRO–UNESCO fellow.

M. C. HARRIS*

J. J. DREIFUSS

Department of Physiology,
Medical School,
Geneva, Switzerland

J. J. LEGROS

Laboratoire de Radioimmunologie,
23, Bd Piercot,
Liege, Belgium

Received July 17; accepted September 18, 1975.

*On leave from the Department of Physiology, University of Birmingham, Birmingham B15 2TT, UK.

1. Swaab, D. F., Pool, C. W., and Nijveldt, F., *J. Neural. Transmission*, **36**, 195–215 (1975).
2. Beleslin, D., Bisset, G. W., Haldar, J., and Polak, R. L., *Proc. R. Soc.*, **B166**, 443–458 (1967).
3. Clark, B. J., and Rocha e Silva, M., Jr., *J. Physiol., Lond.*, **191**, 529–542 (1967).
4. Bisset, G. W., Clark, B. J., and Haldar, J., *J. Physiol., Lond.*, **206**, 711–722 (1970).
5. Wakerley, J. B., Dyball, R. E. J., and Lincoln, D. W., *J. Endocr.*, **57**, 557–558 (1973).
6. Haldar, J., *J. Physiol., Lond.*, **206**, 723–730 (1970).
7. Dreifuss, J. J., *Ann. N.Y. Acad. Sci.*, **248**, 185–201 (1975).
8. Wakerley, J. B., and Lincoln, D. W., *Brain Res.*, **25**, 192–194 (1971).
9. Lincoln, D. W., and Wakerley, J. B., *J. Physiol., Lond.*, **242**, 533–554 (1974).
10. Dreifuss, J. J., and Kelly, J. S., *J. Physiol., Lond.*, **220**, 87–103 (1972).
11. Share, L., in *Handbook of Physiology*, Section 7 (edit. by Knobil, E., and Sawyer, W. H.), **4**, 243–255 (American Physiological Society, Washington DC, 1974).
12. Bisset, G. W., Hilton, S. M., and Poinsner, A. M., *Proc. R. Soc.*, **B166**, 422–442 (1967).
13. Legros, J. J., Stewart, U., Nordmann, J. J., Dreifuss, J. J., and Franchimont, P., *C.r. Séanc. Soc. Biol.*, **165**, 2443–2447 (1971).

Activity of phasic neurosecretory cells during haemorrhage

APPROXIMATELY 25% of the neurones projecting to the neurohypophysis from the paraventricular (PV) and supraoptic (SO) nuclei of the hypothalamus fire phasically and such a pattern is seldom seen in other hypothalamic cells¹. Phasic neurones respond to osmotic stimuli² but are rarely activated by suckling^{1,3}. It is tempting therefore, to associate these cells with the secretion of vasopressin rather than oxytocin. The functional relationship of their phasic pattern to hormone release, however, remains obscure. We have studied phasic PV neurones during haemorrhage, which releases vasopressin, sometimes with small amounts of oxytocin⁴. Haemorrhage evoked changes in phasic units over a period long enough for a detailed analysis of their behaviour. Our results show the importance of pattern

Table 1 Changes in the activity quotient and intraburst frequency of phasic neurosecretory cells during blood removal and blood replacement

	Control values	Change during blood removal				Change during blood replacement			
Time from start (min)		1	2	3	4	1	2	3	4
Activity quotient (Mean $\pm$ s.e.)	0.52 (± 0.06)	+0.09* (± 0.04)	+0.14* (± 0.06)	+0.15† (± 0.05)	+0.24† (± 0.07)	-0.25* (± 0.10)	-0.29† (± 0.06)	-0.47† (± 0.09)	-0.24* (± 0.16)
Intraburst frequency‡ (spikes s ⁻¹) (Mean $\pm$ s.e.)	7.59 (± 1.50)	+0.39 (± 0.29)	+0.49 (± 0.37)	+2.20* (± 0.71)	+5.10* (± 2.13)				

* $P < 0.05$. † $P < 0.01$. Significance levels were determined by the paired t test.

‡ Many of the cells were quiescent during blood replacement and this precluded a statistical analysis of the changes in intraburst frequency during this period.

modulation in phasic neurones and suggest that the mechanism for vasopressin release differs from that described for oxytocin release during suckling^{1,3}.

Lactating female rats (300–350 g), anaesthetised with urethane (1.1 g kg⁻¹), were prepared for intramammary pressure recording¹. Cannulae were introduced into the saphenous artery for monitoring blood pressure and into the right atrium through the jugular vein to remove blood. Animals were then placed in a stereotaxic frame and antidromically identified units were recorded in the PV nucleus by techniques described previously^{1,3}. After locating a phasic unit, we recorded 10–15 min of baseline activity before examining the effect of a 4-ml haemorrhage over a period of 4 min (1.3 ml per 100 g body weight; 15% of blood volume). The first and last 0.5 ml of blood collected were used for plasma osmotic pressure determination and vasopressin assay. Two minutes after haemorrhage, the blood was returned over 4–6 min. The volume of blood taken for assay was replaced with warm isotonic saline. Where possible, the test was repeated for each cell 20–30 min later. Samples were centrifuged and the plasma stored frozen at -20°C until assay. The blood cells were washed in saline and reinjected in a minimum volume of isotonic saline before a further unit was tested.

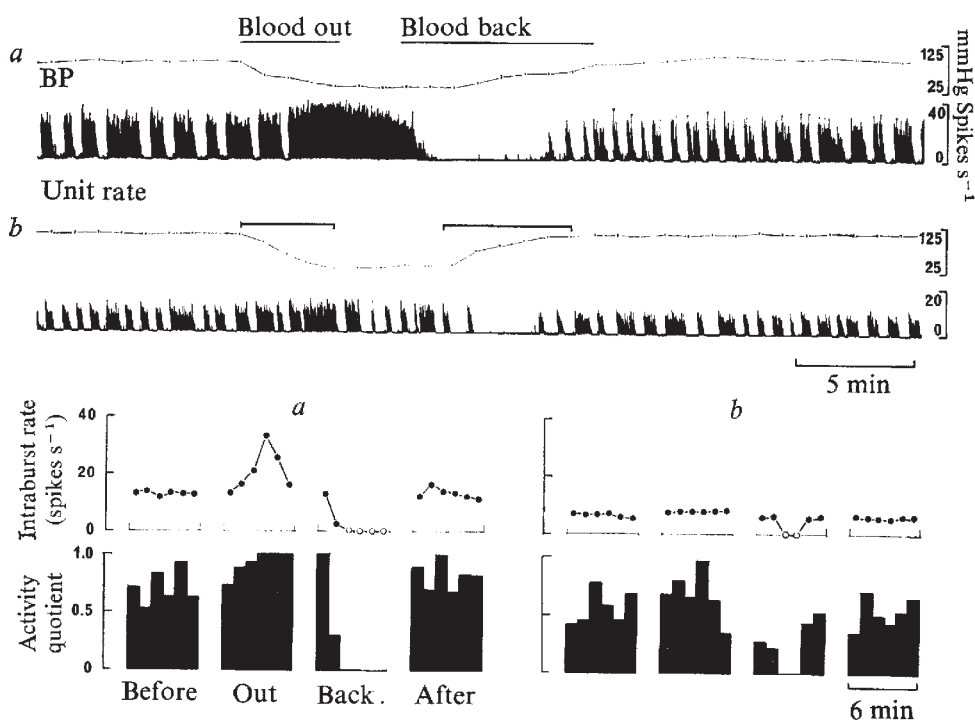
Vasopressin could not be detected in all samples after haemorrhage, probably because of the relative insensitivity of the assay (rat antidiuretic assay: minimum detectable level $> 32 \mu\text{U}$ per ml plasma). In a series of tests, however, the plasma content of antidiuretic activity after blood

removal (median $56 \mu\text{U ml}^{-1}$, $n=21$) was significantly higher ($P < 0.001$, Mann-Whitney U test) than control levels (median $< 32 \mu\text{U ml}^{-1}$, $n=32$). The median value for plasma oxytocin was $< 25 \mu\text{U ml}^{-1}$ both before ($n=23$) and after ($n=17$) haemorrhage, but in two tests oxytocin release was detected. Blood pressure usually dropped from about 100 mmHg to 30–40 mmHg during blood withdrawal and returned to normal levels after blood replacement (Fig. 1). Plasma osmotic pressure (range 295–315 mosmol kg⁻¹) did not change.

Sixty-nine antidromically identified PV units were encountered, of which 15 (22%) fired in a phasic pattern. At histological examination, all were shown to be located in the PV nucleus. The mean duration of the bursts varied considerably between cells (range 6.9–48.6 s) as did the duration of the silent periods (range 9.9–22.2 s); however, for individual neurones the periodicity was quite regular. Ten of the phasic units were recorded throughout the full haemorrhage test. For each minute of the different periods (control, blood out and blood back) the following parameters of spike activity were measured: (1) The activity quotient, Q , which is the total duration of the bursts (s) in 1 min divided by 60; (2) the intraburst frequency, f , representing the mean firing rate within the bursts; and (3) the overall frequency, F , which is the total number of spikes in 1 min divided by 60.

During haemorrhage, the most consistent effect was a prolongation of the bursts (Fig. 1) resulting in a significant increase in Q during each of the first 4 min (Table 1). In

Fig. 1 Records of the firing pattern of 2 phasic cells (*a* and *b*) to show the changes which occurred during the withdrawal and replacement of 4 ml blood. Blood pressure decreased rapidly during blood withdrawal and returned to control level soon after blood replacement. Beneath the records are plotted the changes in firing rate within the bursts (f) and the proportion of time for which the cells were firing (activity quotient, Q). Haemorrhage increased Q for each of the cells tested and one of the cells showed an increase in f . In contrast, blood replacement decreased the values of both Q and f . ○, Period during which the cells were totally inactive.



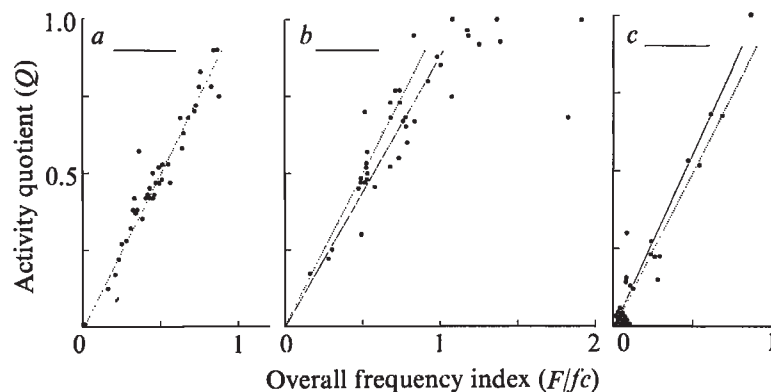


Fig. 2 The relationship between activity quotient (Q) frequency within the bursts (f) and overall frequency (F) during the control period (a), blood withdrawal (b), and blood replacement (c). Q , f and F have been established for each response and they are related by the equations $F = Qf$ or $Q = F/f$. So that the results of all the tests could be considered as a whole, we used the expression F/fc where fc is the mean frequency within the bursts during the control period. If the intraburst frequency remains constant (that is, equal to fc), Q and F/fc are related linearly and all the points fall on the line defined by $Q = F/fc$. If f increases the points fall on the right of the line; if f decreases the points fall on the left of the line. Regression lines have been calculated for the three experimental conditions. For values of Q between 0 and 0.90, the correlation coefficients are highly significant (respectively $r = 0.97$, $r = 0.68$, $r = 0.97$ and $P < 0.001$) and the regression coefficients of the three lines are not significantly different ($P < 0.3$ variance ratio test and Student's t test). $F/fc = 1.01Q - 0.01$ (a); $1.06Q + 0.07$ (b); $0.9Q + 0.01$ (c).

extreme cases, this remained at unity for several minutes (cell a, Fig. 1). Intraburst frequency (f) also changed but less consistently and later: the mean increase was significant during the third and fourth minutes. These events resulted in an increased mean overall frequency (F) from a control level of $3.93 \text{ spikes s}^{-1}$ to $9.60 \text{ spikes s}^{-1}$ in the fourth minute of haemorrhage. Blood replacement produced opposite effects, with reduction in Q resulting from shortened bursts. Many neurones became silent, but occasionally when bursts did occur f decreased. During the third minute F fell to $0.1 \text{ spikes s}^{-1}$. Recovery occurred within a few minutes of the end of the test. In neurones tested twice, the second response was similar to the first.

The relative importance of the change in burst duration and in intraburst frequency is shown in Fig. 2, which correlates Q with a measure of the change in overall frequency, F/fc . For Q up to 0.90 the correlation is highly significant during control period (Fig. 2a), blood removal (Fig. 2b) and blood replacement (Fig. 2c). Furthermore, the slopes of the three regression lines, which provide a measure of the average intraburst frequency, did not differ significantly in the experimental situations. Thus, f did not change appreciably until Q approached unity, that is, when the cell tended to fire continuously.

These changes within individual cells may explain the observations of Arnould *et al.*⁵ in the SO nucleus of the dehydrated monkey: as plasma osmotic pressure increased the predominant types of cells were first slow-firing cells, then phasic cells and finally fast, continuously-firing cells. We may conclude that the phasic pattern is a functional state of activity of a cell which at other times can be silent or firing continually. The significance of this phasic state is possibly that phasic cells are synchronised to produce an episodic discharge of neurohypophyseal hormone. In normal conditions there is no obvious synchronisation of bursts⁶ but overlapping activity within a population of neurones inevitably increases as the duration of bursts increases. The modulation of burst duration, which in our experiments preceded changes in intraburst frequency could provide a precise mechanism for regulating hormone release. An increase of intraburst frequency would further augment hormone release⁷.

These observations do not prove that phasic cells secrete vasopressin, nevertheless we now know that they are activated by hyperosmotic injections², dehydration³, carotid occlusion⁸ and haemorrhage, all of which cause vasopressin release, and that they do not seem to be involved in oxytocin release^{1,3}. We propose, therefore, that, on the basis of their neurophysiological performance, PV and SO neurones projecting to the neurohypophysis may be separated into oxytocinergic cells—characterised by their high frequency synchronous discharge during suckling^{1,3}—and vasopressinergic cells—characterised by a tendency to fire asynchronously in phases, the duration and frequency of which determine the rate of vasopressin release.

We acknowledge the assistance of Mrs B. Deverson.

J.B.W. is a Beit Memorial Fellow, D.A.P. is a recipient of grants from Delegation Generale a la Recherche Scientifique and from Laboratoires Labaz, France.

J. B. WAKERLEY
D. A. POULAIN*
R. E. J. DYBALL
B. A. CROSS

ARC Institute of Animal Physiology,
Babraham, Cambridge CB2 4AT, UK

Received August 14; accepted October 1, 1975.

*Permanent address: Laboratoire de Neurophysiologie, Faculte de Medecine de l'Universite, Place de la Victoire, Bordeaux, France.

¹ Wakerley, J. B., and Lincoln, D. W., *Brain Res.*, **25**, 192–194 (1971); *J. Endocr.*, **57**, 477–493 (1973).

² Hayward, J. N., and Jennings, D. P., *J. Physiol., Lond.*, **232**, 545–572 (1973).

³ Lincoln, D. W., and Wakerley, J. B., *J. Physiol., Lond.*, **242**, 533–554 (1974).

⁴ Fabian, M., Forsling, M. L., Jones, J. J., and Lee, J., *J. Endocr.*, **43**, 175–189 (1969).

⁵ Arnould, E., Vincent, J. D., and Dreifuss, J. J., *Science*, **185**, 535–537 (1974).

⁶ Dyball, R. E. J., and Pountney, P. S., *J. Endocr.*, **56**, 91–98 (1973).

⁷ Cross, B. A., *et al.*, *Rec. Prog. Horm. Res.*, **31**, 243–294 (1975).

⁸ Harris, M. C., Dreifuss, J. J., and Legros, J. J., *Nature*, **258**, 80–82 (1975).

Induction of photoreceptor voltage noise in the dark in *Drosophila* mutant

RANDOMLY occurring, discrete voltage fluctuations (quantum bumps) have been recorded in photoreceptors of several arthropods^{1–4}. It is thought that these bumps sum to produce the receptor potential^{5,6}. We have investigated the relationship between photoexcitation of the visual pigment and the production of quantum bumps using a mutant of *Drosophila melanogaster*—a third chromosome recessive mutant described by Cosens and Manning⁷, in which the electroretinogram decays to baseline during a strong, prolonged stimulus. We will call this mutant the transient receptor potential mutant (*trp*).

Experiments consisted of intracellular recordings from the photoreceptors of intact preparations of white-eyed *trp* mutant (*w; trp*). Figure 1a shows intracellularly recorded responses of a single photoreceptor to various intensities of green light ($\lambda = 540 \text{ nm}$). Figure 1b shows a plot of the intensity-response relationship for the initial transient, and the subsequent steady-state phase of the responses.

In normal flies the steady state persists for the entire duration of the stimulus. The mutant response to dim light is similar to that of the wild type (Fig. 1a, bottom). With increased stimulus intensities, however, the mutant response has a large initial amplitude (as in the wild type), but it decays during stimulus to the same low, steady-state level observed with dim stimuli. The rate of the decay and the final steady-state level attained, vary among individual flies—the response often decaying to the dark-resting potential level.

In the *trp* mutant, voltage noise can be seen superimposed on the response at all the stimulus intensities tested (Fig. 1a). The noise level during the steady-state phase of the mutant res-

ponse is greater than the dark noise level, and it shows little change in amplitude with increases in stimulus intensity. In contrast, in the wild type, the voltage noise amplitude greatly decreases with increased stimulus intensity. The fact that the voltage noise of relatively large amplitude is detected in the mutant response at all intensities rendered the *trp* mutant particularly useful for the present investigation.

The two upper traces of Fig. 2 are intracellular responses to weak, and to 100 times more intense light. The two lower traces are magnifications ($\times 3$) of the portions of the two upper traces indicated by vertical bars. The figure shows that the response to the weak light consists of slow potential fluctuations (noise). Similar noise was recorded and analysed in wild-type *Drosophila*,⁶ where the noise seemed to come from quantum bumps occurring at a low rate. The similarity of the noise recorded here, suggests that the mutant noise also arises from quantum bumps. The mutant response to stronger light (first and third traces), after decaying nearly to the steady-state level (Fig. 2), also displays voltage fluctuations, similar to the bump noise seen in the responses to weak light (second and fourth traces). Since bumps are expected to have their maximum amplitude when elicited from a dark-adapted photoreceptor using dim stimuli, the above result suggests that the bumps elicited in the mutants by strong light also have the same maximum amplitude after the receptor potential has decayed to nearly the steady-state level. It follows, therefore, that the decay of the mutant response during stimulus arises not from the decrease in the bump size, but from a decrease in the number of bumps composing the response.

Figure 1 shows that the steady-state amplitude saturates at a relatively low stimulus level. The saturation of the steady-state amplitude is accompanied by saturation of the response noise characteristics (Fig. 3a). This was demonstrated by calculating the autocovariance $C(\tau)$ (Fig. 3a) from the noise superimposed on the steady-state phases of the responses shown in Fig. 1, using the formula:

$$C(\tau) = [\overline{v(t) - \bar{v}(t)}][\overline{v(t + \tau) - \bar{v}(t)}] \quad (1)$$

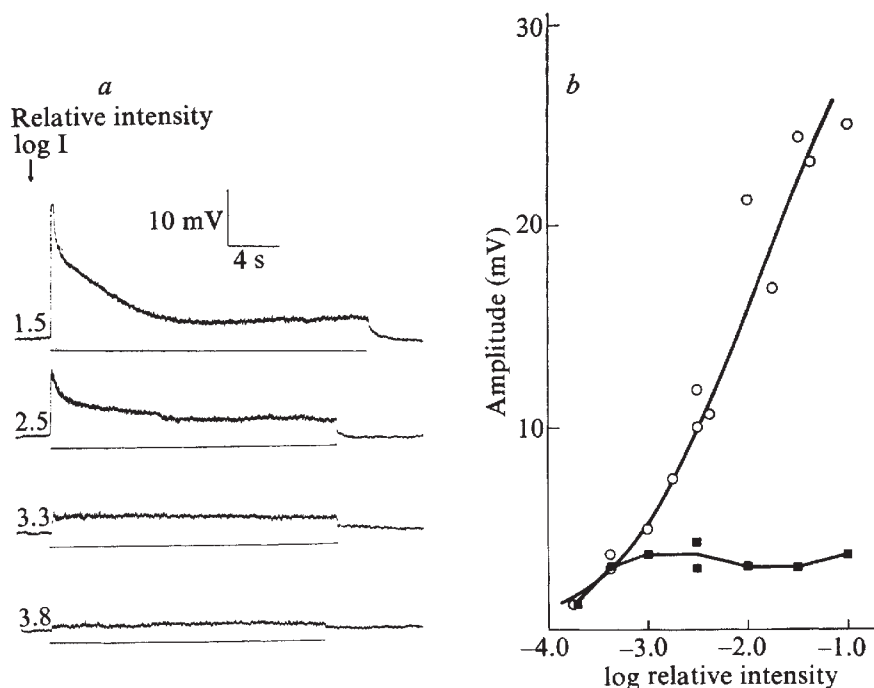
where v is the amplitude of the receptor potential in the steady-state phase, t is the time at which v was measured, and τ is the time lag. The first point, ($\tau = 0$) in the curves is the variance, a measure of noise amplitude. Once the stimulus is strong

enough to be above the saturation intensity for the steady-state phase, the autocovariance shows little dependence on stimulus intensity (Fig. 3a). The autocovariance, calculated from the steady-state response of the same cell at even higher intensities than those shown in Fig. 3a, also showed little dependence on light intensity. Since the steady-state responses to stimuli of very different intensities have nearly the same amplitude (Fig. 1, $\log I = -2.5$ and -1.5) and noise characteristics, that is, nearly the same variance and autocovariance (Fig. 3a), shot-noise analysis⁵ suggests that these responses are produced by superposition of nearly the same number of quantum bumps having similar average bump amplitudes.

In *Drosophila*^{8,9}, as well as in other invertebrates¹⁰⁻¹⁴, an intense coloured stimulus that shifts a substantial amount of the visual pigment (rhodopsin) to its thermally stable state (metarhodopsin), induces an excitatory process manifested by a prolonged depolarising afterpotential (PDA), which far outlasts the stimulus. Photoconverting the pigment back from metarhodopsin to rhodopsin induces an inhibitory process, and depresses or prevents the PDA from occurring. Induction and depression of the PDA in the *trp* mutant are shown in Fig. 3b. The blue light, which shifts rhodopsin to metarhodopsin, depolarises the cell, and the depolarisation is accompanied by an increase in the noise level of the response. When the blue light is switched off, the cell remains depolarised (PDA), and the noise level remains increased during the entire PDA period. The red stimulus shifts the pigment back from metarhodopsin to rhodopsin, depresses the PDA (when the light is switched off), and reduces the noise level to the level before PDA induction. Figure 3a shows the autocovariance calculated from the noise recorded in the dark, during PDA, from the same cell analysed in Fig. 1 and 3a. The similarity between the curves calculated from the light-coincident noise and the PDA noise, suggests that the PDA noise also arises from bumps.

The decay of the receptor potential is possibly caused by light adaptation. Light adaptation has been found to reduce the amplitude of bumps⁵. Figure 2 shows, however, that the bump amplitude does not decrease, while the mutant receptor potential amplitude does decrease, during illumination with stronger stimuli. Furthermore, the application of the shot-noise analysis suggests that, at the saturated steady-state level, the responses to both weaker and stronger stimuli consist of bumps of approximately the same number and amplitude. Both these

Fig. 1 a, Intracellularly recorded responses from photoreceptors of the white-eyed *trp* mutant to various intensities of monochromatic 540-nm green light (half peak bandwidth of 16 nm). The light source was a Xenon arc lamp used in conjunction with Bausch and Lomb high-intensity monochromator. The unattenuated illuminance was 3.0×10^{14} photons $\text{cm}^{-2} \text{s}^{-1}$ at 480 nm, 3.3×10^{14} photons $\text{cm}^{-2} \text{s}^{-1}$ at 520 nm, and 3.6×10^{14} photons $\text{cm}^{-2} \text{s}^{-1}$ at 540 nm. The lines below the responses indicate the duration of the light stimulus. b, A plot of the amplitude-log intensity curves for the transient phase (○) and the steady-state phase (■) of the response. All data points were measured from the cell whose responses are presented in Fig. 1a.



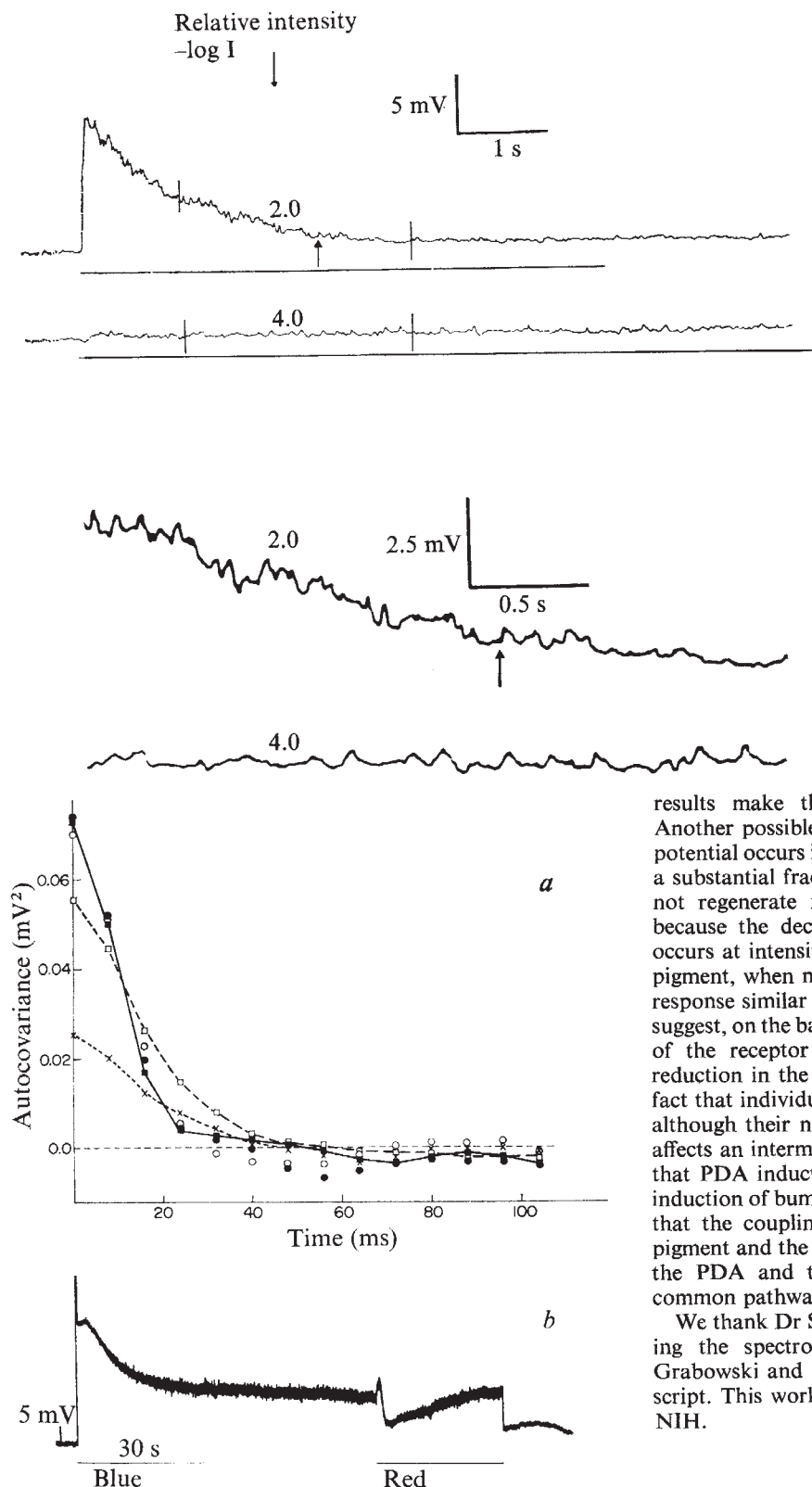


Fig. 3 *a*, Autocovariance functions calculated from dark noise ($\times$), the steady-state responses ($\square$, $-\log I = 3.8$; $\blacksquare$, $-\log I = 2.5$; $\circ$, $-\log I = 1.5$) shown in Fig. 1 and the PDA recorded from the same cell ($\bullet$). The calculations were done from 750 points sampled at 8-ms intervals. The ordinate is the autocovariance $C(\tau)$ in mV² and the abscissa is the time lag τ in ms. The responses were filtered with a high frequency cutoff filter (half power cutoff at 30 Hz) before the analysis. Calculation without filtering gave similar results. *b*, An intracellularly recorded PDA response from the photoreceptor of the mutant *trp*, induced by blue and red stimuli. The stimuli originated from a quartz iodide lamp filtered through Baird Atomic B3 interference filters, having transmission peaks at 480 nm and 600 nm. Illuminances were 4.0×10^{16} photons cm⁻² s⁻¹ and 2.5×10^{16} photons cm⁻² s⁻¹ for the blue and red stimuli, respectively.

Fig. 2 Intracellularly recorded responses from a single photoreceptor of *trp* to the monochromatic 520-nm green light attenuated by 2.0 (first trace) and 4.0 (second trace) neutral density filters. The two lower traces are $\times 3$ magnifications of the marked portions of the two upper traces. The responses were filtered with a high frequency cutoff filter (half power cutoff at 40 Hz), to facilitate observation of bumps. The arrows indicate the approximate time after which, the response to the stronger light shows noise characteristics similar to the response to the weaker light.

results make the light adaptation hypothesis improbable. Another possible explanation is that the decay of the receptor potential occurs in the mutant *trp*, because the stimulus bleaches a substantial fraction of visual pigment, and the pigment does not regenerate normally. This explanation, too, is unlikely because the decay of the potential to the steady-state level occurs at intensities that activate less than 1–3% of the visual pigment, when measured spectrophotometrically or by using a response similar to the early receptor potential¹⁵. We therefore suggest, on the basis of the results shown in Fig. 2, that the decay of the receptor potential during a stimulus arises from a reduction in the quantum efficiency of bump production. The fact that individual bumps in the mutant appear to be normal, although their number is reduced, suggests that the mutation affects an intermediate step of phototransduction, and the fact that PDA induction in the *trp* mutant is accompanied by the induction of bump noise after the cessation of stimulus, suggests that the coupling between the photoexcitation of the visual pigment and the production of the bump is not direct, and that the PDA and the stimulus-coincident response share some common pathway.

We thank Dr S. E. Ostroy and Mrs Meegan Wilson for making the spectrophotometric measurements and Drs S. R. Grabowski and S. Nakajima for critical reading of the manuscript. This work was supported by grants from NSF and the NIH.

BARUCH MINKE
CHUN-FANG WU
WILLIAM L. PAK

Department of Biological Sciences,
Purdue University,
West Lafayette, Indiana 47907

Received July 21; accepted September 18, 1975.

- Yeandle, S., thesis, Johns Hopkins University (1957).
- Kirschfeld, K., in *The Functional Organization of the Compound Eye*, 291–307 (edit. by Bernhard, C. G.) (Pergamon, Oxford, 1966).
- Sholes, J., *Cold Spring Harb. Symp. quant. Biol.*, **30**, 517–527 (1965).
- Fouries, M. G. F., and Yeandle, S., *J. gen. Physiol.*, **47**, 443–463 (1964).
- Dodge, F. A., Knight, B. W., and Toyoda, J., *Science*, **160**, 88–90 (1968).
- Wu, C.-F., and Pak, W. L., *J. gen. Physiol.*, **66**, 149–168 (1975).
- Cosens, D., and Manning, A., *Nature*, **224**, 285–287 (1969).
- Minke, B., Wu, C.-F., and Pak, W. L., *J. comp. Physiol.*, **98**, 345–355 (1975).
- Cosens, D., and Briscoe, D., *J. Insect Physiol.*, **18**, 627–632 (1972).
- Nolte, J., and Brown, J. E., *J. gen. Physiol.*, **59**, 186–200 (1972).

- ¹¹ Hillman, P., Hochstein, S., and Minke, B., *Science*, **175**, 1486–1488 (1972).
¹² Hochstein, S., Minke, B., and Hillman, P., *J. gen. Physiol.*, **62**, 105–128 (1973).
¹³ Minke, B., Hochstein, S., and Hillman, P., *J. gen. Physiol.*, **62**, 787–791 (1973).
¹⁴ Muijsers, H., Leutscher-Hazelhoff, J. T., Stavenga, D. G., and Kuiper, J. E., *Nature*, **254**, 520–522 (1975).
¹⁵ Pak, W. L., and Lidington, K. J., *J. gen. Physiol.*, **63**, 740–756 (1974).

Possible contribution of ionic clustering to molecular packing of collagen

An analysis of the primary structure of the $\alpha 1$ chain of a collagen demonstrated that electrostatic and hydrophobic interactions between collagen molecules were maximal when the rod-like molecules were staggered by $234n$ amino acid residues, where n is 0, 1, 2, 3 or 4 (ref. 1). It was not possible, however, to determine what value of n was most likely. This information would help to select from among possible molecular packing models^{2,3}. Doyle *et al.*⁴ re-examined the electrostatic interactions in an attempt to determine n . Since amino acid side chains of opposite charge frequently occur close to each other in the sequence¹, they considered that these potential intrachain ion pairs might function as dipoles with approximately the same direction in the molecule as in the sequence. If antiparallel dipoles on adjacent molecules were attractive (Fig. 1a) and parallel dipoles were repulsive, they found that the net number of attractions was maximal at $n = 1$ and negative at other values of n . They concluded that dipole-dipole interactions were important in stabilising the native fibril and that the Smith microfibril⁵ was favoured over other packing models since it is essentially a helix of collagen molecules staggered by 234 residues between nearest neighbours.

Although we find the assumptions made by Doyle *et al.*⁴ difficult to accept, they were apparently able to discriminate from among possible values of n . This suggests that the reason should be more carefully examined. We discuss here other aspects that may be important and propose as an alternative concept that ionic clustering may contribute significantly to the stabilisation of collagen fibril structure.

First, interactions between dipoles cannot be estimated when the location of the charges is not well defined. This point is of particular importance in the case of collagen. The charges are at the ends of side chains, which may assume many configurations, and there are generally three sets of charges at any level since there are three similar or identical chains in the molecule. Therefore, neighbouring charges of opposite sign in a single chain will not necessarily form a dipole in an actual structure, and even if dipoles are formed, the contributing ions and their orientation would be difficult to predict. A related conclusion is that the ions would be able to arrange themselves so as to minimise electrostatic repulsion, which then would not be a major factor, contrary to the assumption of Doyle *et al.*⁴

Second, there is information in the sequence, not explicitly used by Doyle *et al.*⁴, that explains their result without recourse to a consideration of dipole-dipole interactions. Not only are amino acid side chains of opposite charge found close to each other in the sequence much more often than would occur by chance, but glutamic acid and arginine are not equally distributed between position X and Y in the collagen triplet Gly-X-Y, with the result that about three out of four positive charges precede glycine and about three out of four negative charges follow glycine in the sequence. Therefore triplets of the form Y-Gly-X and Gly-X-Y, where Y has a positively charged side chain and X has a negatively charged side chain, are common. Of the fifty 'dipoles' considered by Doyle *et al.*⁴, fifteen are of the former type and thirteen are of the latter type. This geometry suggests an arrangement (Fig. 1b) of four alternating unit charges approximately in a line that could result in cooperative electrostatic interactions with a lower potential energy than two separated ion pairs and a more favourable stereochemical relationship than the two 'dipoles' in Fig. 1a. An examination of molecular models shows that such an arrangement is possible

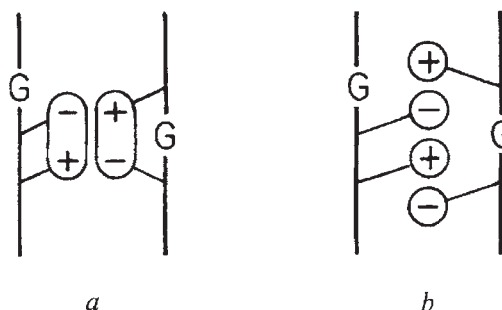


Fig. 1 a, Antiparallel dipoles between collagen molecules considered to be attractive by Doyle *et al.*⁴. b, An alternative arrangement of the ions to form a line of alternating charges. G is glycine. The positive and negative ions show two characteristic orders of charged amino acids in the triplet sequence of collagen chains.

because of the separation of the one pair by a glycine residue. Furthermore, the arrangement provides a reason for the unusual distribution of glutamic acid and arginine. Similar arrangements of other opposing antiparallel charge pairs can be devised. This approach to the location of ionic groups leads to the conclusion reached by Doyle *et al.*⁴ that $n = 1$, if the same scoring system is used. If, however, the possibility of repulsive interactions is de-emphasised and other arrangements of charges are allowed, as we argue, a most probable value of n can no longer be selected with confidence by this analysis.

The considerations discussed here suggest a concept that may help in understanding how collagen fibrils are stabilised. The argument is as follows. Since the collagen molecule has three similar or identical chains as already noted, and since more than one chain per molecule may contribute groups to interacting edges between molecules, an arrangement of the type shown in Fig. 1b, if viewed in three dimensions, could involve additional ionic groups. Furthermore, there are often more than two ionic groups near each other in the sequence, and it is possible that an interesting region may involve more than two molecules. Therefore, clusters of ions may exist. Schematic representations of the Smith microfibril suggest that as many as 16–20 ions could cluster in ordered arrangement. These would be expected to approximate the properties of an ionic crystal and contribute some measure of stability to the collagen fibril structure analogous to crystal field energy. This could be an especially important contribution if the clusters were in the interior of the Smith microfibril, or one of the similar alternative structures, since the movement of water, other small molecules, and amino acid side chains would be restricted, increasing the likelihood of cooperative ordering and depolarisation. A more complete analysis may settle some of the details of the molecular packing of collagen, such as the value of n , but this has not yet been done.

The principle of ordered ionic clustering proposed here may apply generally to supramolecular structures, which create extended regions where movement is restricted. Guss *et al.*⁶ have found an example by X-ray diffraction of a helical form of hyaluronic acid which has a line of alternating charges of opposite sign down the centre of the helix.

KARL A. PIEZ
DENNIS A. TORCHIA

Laboratory of Biochemistry,
National Institute of Dental Research,
Bethesda, Maryland 20014

Received April 2; accepted September 12, 1975.

- ¹ Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A., and Woodhead-Galloway, J., *J. molec. Biol.*, **79**, 137–148 (1973).
² Doyle, B. B., *et al.*, *Proc. R. Soc.*, **B186**, 67–74 (1974).
³ Piez, K. A., and Miller, A., *J. Supramolec. Struct.*, **2**, 121–137 (1974).
⁴ Doyle, B. B., *et al.*, *Biochem. biophys. Res. Commun.*, **60**, 858–864 (1974).
⁵ Smith, J. W., *Nature*, **219**, 157 (1968).
⁶ Guss, J. W., *et al.*, *J. molec. Biol.*, **95**, 359–384 (1975).

Mobile charge carriers in photosynthesis

CURRENT mechanistic models of energy transduction in photosynthesis, envision that the energy of the photons collected by the bulk antenna chlorophyll, is funnelled to special sites called reaction centres, where the primary charge separation events occur¹. If the primary oxidant and reductant are separated physically by only a few, or few tens of angstroms, changes in the dielectric properties should become evident². Several laboratories have sought such evidence with equivocal results³⁻⁶, although one group, using methods similar to ours, has reported qualitative, positive results⁷.

We report here direct confirmation of the presence and trapping of charge carriers of both signs, their mobilities and relative quantum yields, and an estimate of the distance between the separated units of charge.

Our experiments measured the light-induced changes in the real and imaginary parts of the complex dielectric constant, $\epsilon = \epsilon' + i\epsilon''$, of photosynthetic samples at the microwave frequency 9 GHz. Changes in the imaginary part of the dielectric constant, ϵ'' , reflect the losses or photoconductivity, $\Delta\sigma$, whereas changes in the real part, ϵ' , can be related to light-induced changes in the polarisability, α , from which a distance can be estimated⁸⁻¹⁰. The signs and Hall mobilities of the carriers were determined by measurements of the light-induced Faraday rotation. The apparatus consisted of a phase coherent microwave instrument including a bimodal cylindrical transmission cavity¹¹, the design and performance of an earlier version have been reported previously¹².

The biological samples consisted of films of whole and broken chloroplasts from plants and algae, and chromatophores from photosynthetic bacteria. The samples were prepared according to well established experimental procedures^{13,14}, and the films were obtained by evaporation, in a mild air flow, of aqueous suspensions of the photosynthesis systems. Partial dehydration of the samples did not alter the primary photo-events, which were monitored by the measurement of light-induced electron paramagnetic resonance signals^{15,16}, and of light-induced absorbance changes, indicating bleaching of the reaction centre pigments^{1,16}. The films were deposited on microscope cover slips, and were located, in the microwave cavity, at the antinode of the microwave electric field, by means of styrofoam supports. Light pulses obtained from a Xenon flash tube or a ruby laser were fed into the cavity by means of quartz light pipes. Absolute light intensities were measured at the sample location by fast silicon light detectors, with calibrations traceable to the USA National Bureau of Standards.

Our primary observables were the light-induced changes in the microwave power coupled through the bimodal transmission cavity, which are related to the photoconductivity⁸. Accompanying these, there may be changes in the real part of the dielectric constant, which are reflected as changes in the resonance frequency of the cavity⁹. On application of an external magnetic field, with particular adjustments of the bimodal cavity, the power coupled through the cavity is related to the Faraday rotation of the sample¹¹. When the cavity is imperfectly adjusted the photoconductivity and Faraday rotation signals are superimposed, but can be separated.

Figure 1a is an example of a photoconductivity signal, $\Delta\sigma$, from a sample of chloroplasts of *Spinacia oleracea*. As $\Delta\sigma = \Delta n q \mu$, where Δn is the change in number of carriers of charge, q , and mobility, μ , it is necessary to determine μ to obtain values of Δn ; otherwise the data are only qualitative⁷. Light-induced changes in carrier mobility were negligible, and thus did not contribute significantly to the observed photoconductivity. Estimation of the quantum yield for carrier generation, $QY = \Delta n/\text{quanta absorbed}$, required knowledge of the changes in carrier concentration Δn , and the quanta absorbed by the sample. The Hall mobility, $\mu_H = \theta_H/B$, can be obtained from measurements of the Faraday rotation, where

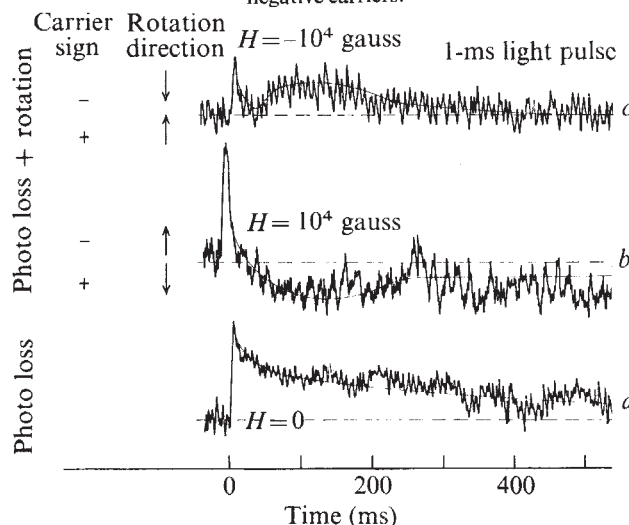
θ_H is the Hall angle, or angular rotation of the carrier current on application of an external magnetic field of intensity B . Figures 1b and 1c are examples of Faraday rotation signals, for a value of $B = 10^4$ gauss, superposed on photoconductivity signals. In Fig. 1b, the settings were such that a negative carrier would produce an upward deflection, positive carriers would produce a downward deflection. These Faraday rotation signals add algebraically to the photoconductivity signal of Fig. 1a. The early temporal component in Fig. 1b is larger in magnitude than the corresponding portion of Fig. 1a, thus establishing its origins in negative carriers. The later temporal components in Fig. 1b are of opposite sign to the early component, and subtract from the photoconductivity signal of Fig. 1a. They are thus attributed to positive carriers. In Fig. 1c, the experiment was repeated with the direction of the external magnetic field reversed. The field-dependent components reverse their deflection directions, and the early temporal components subtracts from the photoconductivity signal of Fig. 1a, whereas the latter components add to it. The sum of Fig. 1b and 1c removes the magnetic field-dependent signals, while their difference removes the photoconductivity signal. The reversal of the field-dependent signals on reversal of the sign of the external magnetic field, established the origins of these signals as Faraday rotations.

The Hall mobilities as calculated from Fig. 1 are of the order of $1 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. Using this value of μ , we calculated the number of positive carriers as 10^{13} cm^{-3} for saturating intensities of actinic light, and quantum yields around 1% for non-saturating illumination levels.

Data, similar to those shown in Fig. 1, have been obtained from leaves and chloroplasts of other green plants and algae, and from chromatophore fragments of the photosynthetic bacterium *Rhodospseudomonas spheroides*. Notably, the non-photosynthetic mutant of this organism, PM-8, gave rise to signals one to two orders smaller in magnitude, than those exhibited by the photosynthetically active varieties, and did not exhibit the sign reversal in the Faraday rotation experiments.

Figure 2 shows the wavelength dependence of the microwave photoconductivity signals for a sample of spinach chloroplasts similar to that from which the data of Fig. 1 were obtained. This dependence generally parallels that for photosynthesis¹⁸, aside from an increase towards the longer wavelength, where the reaction centre pigments absorb. A similar long wavelength

Fig. 1. Microwave photoconductivity and Faraday rotation signals from a spinach chloroplast film. The photoconductivity signal, trace *a*, exhibits both fast and slow components. In *b* a Faraday rotation signal adds algebraically to the photoconductivity of *a* so that the early temporal components attributable to negative carriers add while the later components subtract. For the opposite direction of magnetic field, *c*, the Faraday rotation signals reverse sign while the photoconductivity maintains the original sign, thus leading to a subtraction of the signal for the negative carriers.



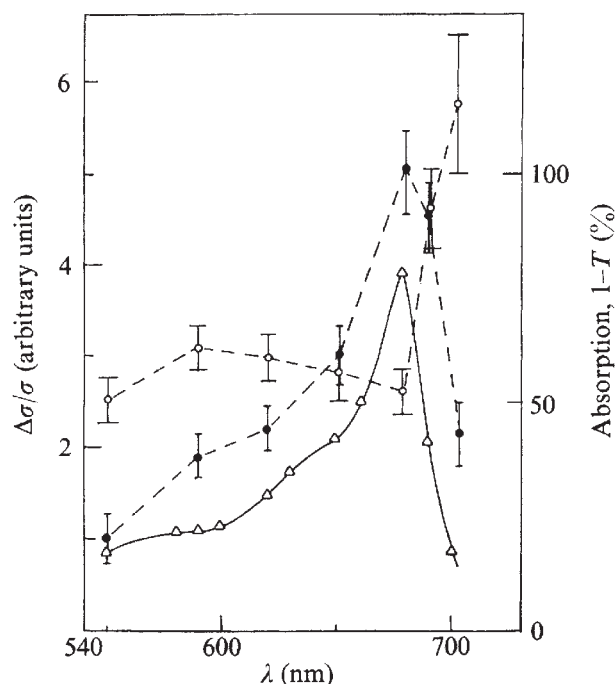


Fig. 2. Action spectrum and relative quantum yield for the microwave photoconductivity in a spinach chloroplast film. Δ , Absorption spectrum of the sample; $\bullet$, action spectrum, $\circ$, relative quantum yield. The points are normalised to constant incident light intensity at 550 nm.

increase was also observed in the photosynthetic bacteria.

Superposition of pulsed light on steady background illumination, is a standard procedure in semiconductor physics to study trapping processes¹⁰. Such procedures were used in our experiments; they provided evidence for the trapping of carriers of both signs, and facilitated a rough determination of the trap concentration of $\sim 10^{13} \text{ cm}^{-3}$. The number of reaction centres, estimated from the chlorophyll content of the sample, was also $\sim 10^{13} \text{ cm}^{-3}$. Arrhenius plots of the temperature dependency of the photoconductivity signals and their decay times, gave activation enthalpies of 0.3 eV.

Light-induced changes in the real part of the dielectric constant, $\Delta\epsilon'$, of $\sim 10^{-5}$, were observed. Changes in the dielectric constant can be related to changes in volume polarisability, α , using the Clausius-Mossotti relationship¹⁰. From this value of $\Delta\epsilon'$, we calculated a value of $\Delta\alpha$ of 10^{-20} cm^3 . Assuming that α approximates the volume over which the charges are separated, $\alpha^{1/3} = 3 \times 10^{-7} \text{ cm}$, represents a separation length. This value is comparable with the presumed size of the pigment-protein complex of the reaction centre^{19,20}.

In the bacterial chromatophores the light-induced ESR signals exhibit decay half-life regimes of 30–50 ms, and of several seconds; the positive carriers exhibit very similar kinetic components. The reaction centreless mutant, PM-8, exhibits neither Faraday rotation, nor photo-induced ESR signals. Titration of the chromatophores with ferricyanide to maximum dark ESR signal intensity, concurrently eliminates the slow components of the photoconductivity signals. Taken together, these observations point to a common origin for the ESR and positive carrier photoconductivity signals.

The positive carrier component is thus interpreted as originating in the same species that produced the ESR signal I, the dimeric chlorophyll cation radical^{20,21}, and we suggest that this species behaves as a mobile hole (over a volume of $30^\circ \text{ \AA}^3$), when observed at microwave frequencies. The negative carrier component of the photoconductivity (few ms range) is tentatively interpreted as arising from electrons thermally released from the primary acceptors (the reverse process), and its decay as resulting from charge transfer to secondary acceptors; because of their larger energy gap, they would not contribute significantly to the population of the conducting state. Rigorous interpre-

tation of the observed activation enthalpies is seriously hindered by the fact that they may represent a combination of activations for both carrier types, and it is not possible to separate their contributions from the available data.

The general behaviour, therefore, can be understood in terms of photoliberated charges migrating for short distances through a dielectric medium. The microwave Hall mobilities are incompatible with charge transport through conduction bands; rather, they are similar to those expected for tunnelling or hopping processes¹⁰.

These observations and interpretations, which will be elaborated fully elsewhere, strongly support the model in which the primary charge separating events occur in the reaction centres in photosynthesis. The fact that such signals are not observed in the reaction-centreless mutant of *R. spheroides*, together with stringent controls, and other evidence not reported here, rules out an artefactual source of the signals. The action spectra for the microwave photoconductivity, resembling, as they do, those for photosynthesis, point to a close connection between the two processes. The number of traps, as well as the maximum number of free carriers, approximates the number of reaction centres, thus connecting the observations with the structure of the photosynthetic unit.

This work was sponsored by the US Energy Research and Development Administration.

ROBERTO A. BOGOMOLNI*
MELVIN P. KLEIN

Laboratory of Chemical Biodynamics,
Lawrence Berkeley Laboratory,
University of California,
Berkeley, California 92740

Received January 16; accepted September 19, 1975.

* Present address: Cardiovascular Research Institute, University of California Medical Center, San Francisco, California.

1. Clayton, R. K., *Adv. chem. Phys.* **19**, 353–378 (1971).
2. Katz, E., *Photosynthesis in Plants*, (Iowa State College Press, Ames, Iowa, 1949).
3. Arnold, W., and Sherwood, H., *Proc. natn. Acad. Sci. U.S.A.*, **43**, 105–114 (1957).
4. Arnold, W., and Clayton, R. K., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 769–776 (1960).
5. McCree, K. J., *Biochim. biophys. Acta*, **102**, 96–102 (1965).
6. Litvin, F. F., and Zvalinskii, V. I., *Biofizika*, **16**, 420–429 (1971).
7. Blyumenfeld, L. A., et al., *Dokl. Akad. Nauk. USSR*, **193**, 700–702 (1970).
8. Portis, A. M., *Phys. Chem. Solids*, **8**, 326–329 (1959).
9. Ginzton, E. L., *Microwave Measurements* (McGraw-Hill, New York, 1957).
10. Guimann, F., and Lyons, L. E., *Organic Semiconductors* (John Wiley, New York, 1967).
11. Portis, A. M., and Teaney, D., *J. appl. Phys.*, **29**, 1692–1698 (1958).
12. Teaney, D., Klein, M. P., and Portis, A. M., *Rev. Sci. Instr.*, **32**, 721–729 (1961).
13. Sun, A. S. K., and Sauer, K., *Biochim. biophys. Acta*, **234**, 399–414 (1971).
14. Reed, D. W., and Clayton, R. K., *Biochem. biophys. Res. Commun.*, **30**, 471–475 (1968).
15. Bolton, J. R., and Cost, K., *Photochem. Photobiol.*, **18**, 417–421 (1973).
16. Baker, R. A., and Weaver, E. C., *Photochem. Photobiol.*, **18**, 237–244 (1973).
17. Siström, W. R., and Clayton, R. K., *Biochim. biophys. Acta*, **88**, 61–73 (1964).
18. Rabinowitch, E., *Photosynthesis*, 2, Part 1, 1083 (Interscience, New York, 1951).
19. Feher, G., *Photochem. Photobiol.*, **14**, 373–387 (1971).
20. Feher, G., Hoff, A. J., Isaacson, R. A., and McElroy, J. D., *Abstr. Biophys. Soc.*, **17**, 611 (a) (1973).
21. Norris, J. R., Druryan, M. E., and Katz, J. J., *J. Am. chem. Soc.*, **95**, 1680–1682 (1973).

Kinetic evidence for a conformational transition in rhodopsin

AFTER light absorption, rhodopsin decays through a series of spontaneous reactions^{1–10}, the kinetics of which have been the object of considerable study. In a variety of conditions the decay kinetics of bathorhodopsin (prelumirhodopsin), lumirhodopsin, and metarhodopsin I (meta I) have been found to be non-first order, but are consistent with a set of parallel, independent first-order reactions^{2–10}. These kinetics have been assumed to result from the existence of multiple forms of each of these intermediates, and it has been proposed that rhodopsin itself exists in different forms or different environments in some conditions⁷. The kinetics of meta I $\rightarrow$ meta II in both dodecyltrimethylammonium bromide- and digitonin-solubilised phospholipid-free rhodopsin are also consistent with two forms of meta I which decay independently (J. G. S. and E. O. Plante, unpublished). We show here that the effect of temperature changes on these kinetics is consistent with the hypothesis that

rhodopsin exists in two forms in equilibrium resulting from the accessibility of two stable conformational states separated by a small difference in free energy.

We have studied the kinetics of rhodopsin decay reactions using flash photolysis of detergent-solubilised rhodopsin containing less than 0.2 mol phospholipid per mol rhodopsin. The kinetics of meta I $\rightarrow$ meta II were determined from absorbance measurements at 380 nm and of lumirhodopsin $\rightarrow$ meta I from absorbance measurements at about 415 nm, which is near the isosbestic point of meta I and meta II. In each case the absorbance against time function could be fitted by a sum of two exponential decay functions of the form $\Sigma A_i \exp(-t/\tau_i)$. We used a phospholipid-free preparation so that we could exclude phospholipid heterogeneity of the digitonin micelle as a source of multiexponential kinetics.

We have assumed that these kinetics result from the independent decay of two forms of each of these species, and thus that the amplitudes of the two exponential terms are proportional to the initial concentrations of these two forms. Figure 1 shows that for meta I $\rightarrow$ meta II a change in temperature from 20 to 37 °C results in a change in the ratio of these two amplitudes, but not in their sum (Fig. 1a and b). Furthermore, this change is reversible (Fig. 1c). Such kinetics are caused by two components which are interconvertible, but only on a time scale which is long compared with the time constants for decay of meta I. Thus, these two components exist in equilibrium, but this equilibrium is established before the appearance of meta I.

We also found that the kinetics of decay of the earlier intermediate, lumirhodopsin, at 20 and 30 °C were consistent with double-exponential kinetics with approximately the same relative amplitudes as those determined from the meta I decay kinetics at these two temperatures. Presumably, the decay kinetics of bathorhodopsin, the earliest of the intermediates, would also be double exponential with the same ratio of amplitudes in these conditions, but we have as yet made no attempt to test this experimentally. Bathorhodopsin does, however, decay with double-exponential kinetics in rod outer segment (ROS) suspensions⁶. Thus, all of these intermediates display the same type of double-exponential decay kinetics. This suggests that rhodopsin itself exists in two forms before photolysis.

According to this interpretation, the ratio of the two amplitudes determined from the meta I $\rightarrow$ meta II kinetics is equal to the equilibrium constant for the transition between these two forms of rhodopsin. The values of the thermodynamic

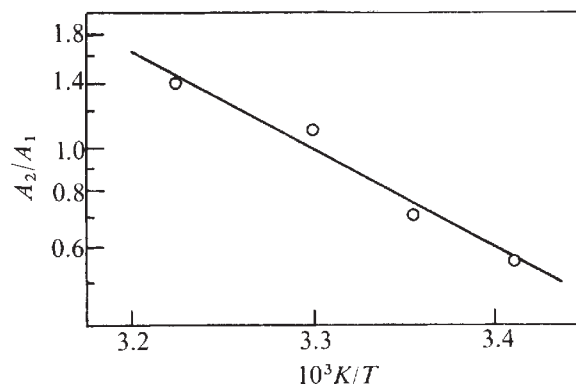


Fig. 2 Van't Hoff plot for the proposed reaction $R_1 = R_2$ over the range 20–37 °C. At each temperature, A_1 and A_2 were determined as described in Fig. 1. A straight line was fitted to the data points by linear least squares, giving $\Delta H^\circ = 9.8$ kcalorie and $\Delta S^\circ = 32$ e.u.

parameters for this hypothetical transition were calculated from the temperature dependence of the equilibrium constant in the range 20–37 °C (Fig. 2). For phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, pH $\approx$ 6, $\Delta H^\circ = 9.8$ kcalorie, $\Delta S^\circ = 32$ e.u., and at 37 °C, $\Delta G^\circ = -0.2$ kcalorie. These values are of approximately the same magnitude as those reported for transitions between some conformational substates of α -chymotrypsin^{11,12}. Two components in these kinetics could also be due to the availability of two different environments in the digitonin micelle, but we have recently obtained similar results with ROS solubilised with cetyltrimethylammonium bromide, Emulphogene BC720, Amonyx-LO and with detergent-free suspensions of sonicated ROS. Thus, it seems likely that the heterogeneity inferred from these kinetics resides in the state of the protein itself.

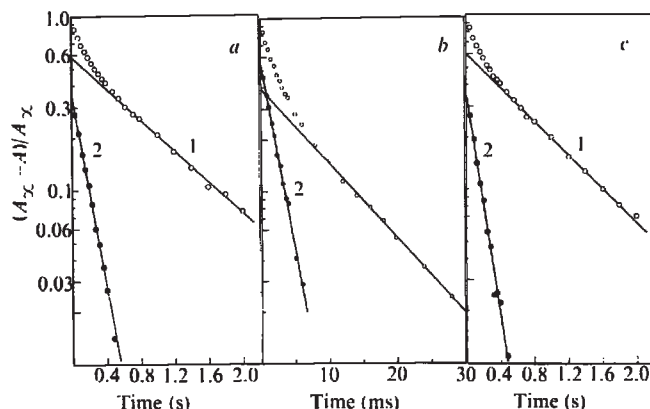
This work was supported by grants from the NIH, NSF and ERDA.

JAMES G. STEWART
BARBARA N. BAKER
THEODORE P. WILLIAMS

*Institute of Molecular Biophysics,
The Florida State University,
Tallahassee, Florida 32306*

Received June 4; accepted August 21, 1975.

Fig. 1 Kinetics of change in A_{380} with phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, pH $\approx$ 6. A_{380} , as a function of time, is relative to the preflash absorbance of the solution. Time constants and amplitudes were extracted from the data by linear least squares fitting and the 'peeling off' procedure. $\circ$, $(A_\infty - A)/A_\infty$; $\bullet$, difference between these points and line 1. a, Sample photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.62$. b, Sample photolysed at 37 °C; $A_1 + A_2 = 0.96$, $A_2/A_1 = 1.4$. c, Sample held at 37 °C for 3 min, and photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.59$.



- Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G., *J. gen. Physiol.*, **47**, 215–240 (1963).
- Linschitz, H., Wulff, V. J., Adams, R. G., and Abrahamson, E. W., *Archs biochem. Biophys.*, **68**, 233–236 (1957).
- Wulff, V. J., Adams, R. G., Linschitz, H., and Abrahamson, E. W., *Ann. N.Y. Acad. Sci.*, **74**, 281–290 (1958).
- Abrahamson, E. W., Marquisee, J., Gavuzzi, P., and Roubie, J., *Z. Elektrochem.*, **64**, 177–180 (1960).
- Grellmann, K.-H., Livingston, R., and Pratt, D., *Nature*, **193**, 1258–1260 (1962).
- Pratt, D. C., Livingston, R., and Grellmann, K.-H., *Photochem. Photobiol.*, **3**, 121–127 (1964).
- Marquisee, J., thesis, Case Western Reserve Univ., Cleveland, Ohio (1966).
- Rapp, J., Wiesenfeld, J. R., and Abrahamson, E. W., *Biochim. biophys. Acta*, **201**, 119–130 (1970).
- Williams, T. P., Baker, B. N., and McDowell, J. H., *Expl Eye Res.*, **18**, 69–75 (1974).
- Applebury, M. L., Zuckerman, D. M., Lamola, A. A., and Jovin, T. M., *Biochemistry*, **13**, 3448–3458 (1974).
- Lumry, R., and Biltonen, R., in *Structure and Stability of Biological Macromolecules* (edit. by Timasheff, S., and Fasman, F.), (Dekker, New York, 1969).
- Kim, Y. D., and Lumry, R., *J. Am. chem. Soc.*, **93**, 1003–1013 (1971).

Erratum

In the article "Seismic evidence for local undulation of olivine-spinel phase boundary", by S. K. Dey-Sarkar and R. A. Wiggins (*Nature*, **257**, 572; 1975) the last two sentences of the first paragraph should read: While analysing seismograms between 14 and 18°, a group of anomalous data was observed localised in a specific region which could be explained only by a local undulation of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.

obituary

John Morrison Barnes, CBE, Director of the MRC Toxicology Unit, Carshalton, died after a short illness on September 24 at the age of 62.

Dr Barnes studied medicine at the Universities of Cambridge and Sheffield and graduated in 1936. He received his early training in research in the laboratory of Howard Florey at Oxford, where he studied lymphocytes and also carried out very important initial toxicity tests on penicillin. He then collaborated with J. Trueta on work related to war injuries, including research on limb ischaemia, the absorption of bacterial toxins from tissues and the treatment of burns. He joined the Royal Army Medical Corps in 1942 and spent the next three years at the Defence Establishment at Porton, with which he remained closely associated as an adviser for the rest of his life. In 1947 he was responsible for setting up the MRC Toxicology Unit at Carshalton which, from very small beginnings, grew under his directorship to become one of the largest establishments of the MRC. The guiding principle of nearly all the research at the Toxicology Unit was derived from the concept of Claude Bernard that toxins could be used to elucidate physiological processes coupled with the ideas of the biochemical lesion and of lethal synthesis, due to Peters. Many toxic substances were studied, Barnes himself being particularly interested in the toxicology of beryllium, organophosphorus compounds, organotin com-

pounds, nitrosamines, aflatoxin and carbon disulphide. Barnes was perhaps best known internationally for his work on organophosphorus and carbamate pesticides. The Unit became a World Health Organisation International Reference Centre for the Evaluation of the Toxicity of Pesticides in 1967 and Barnes advised the Ministry of Agriculture on medical aspects of pesticides for many years. He produced an invaluable guide, *Toxic Hazards of Certain Pesticides to Man* (WHO Monograph 16). Because of his impressive depth of scientific knowledge combined with his great practical common sense, Barnes was uniquely in demand as an advisor on all aspects of possible hazards from environmental and occupational exposure to toxic chemicals. He was a member of numerous Governmental and other advisory committees including the British National Committee for Problems of the Environment. He served on the editorial boards of several scientific journals and was made Visiting Professor of Pharmacology at King's College, London in 1967. Barnes had considerable skill as a laboratory bench worker but little opportunity to exercise it in his later years because of the punishing load of advisory and administrative work that he willingly undertook. His unobtrusive help and guidance was greatly valued by his colleagues and had he been less unselfish, his output of original published work could have been much greater.

John Barnes contributed more to the establishment of toxicology as a scientific discipline than anyone in this country and perhaps in the world.

P. N. Magee

Professor J. C. Pócza, Head of the Department of Structure Research of the Research Institute for Technical Physics of the Hungarian Academy of Sciences, Budapest, died on September 10. Born in 1915, he graduated from the University of Szeged in 1938 and obtained his Ph.D. at Budapest University in 1945.

Professor Pócza first worked on structure determination by X-ray diffraction and then, as assistant at the Institute of Nuclear Physics, took part in the first lunar-echo measurements, carried out under the direction of Professor Z. Bay.

He entered the field of thin film physics in 1954, studying the thin-film growth processes by electron microscopic *in situ* methods. He was a member of the International Committee on Thin Films, a member of the Editorial Board of Thin Solid Films, and was the Chairman of the 3rd International Thin Film Conference held in Budapest this summer.

For many years he was the deputy general secretary of the Hungarian Eötvös Loránd Physical Society and a member of the Hungarian National Committee of IUVESTA.

J. C. Anderson

announcements

Award

The 1975 **Premio Modesto Panetti** has been awarded to **R. S. Rivlin** for his work in the field of mechanics.

Appointments

J. L. Harper, J. S. Sawyer and **E. A. Vincent** have been appointed new members of the Natural Environment Research Council.

International meetings

November 12, **A new look at pollination**, Kent (The Principal's Secretary, Wye College, Ashford, Kent, UK).

Person to Person

London-Rio de Janeiro exchange.

London house available for one year from about February 1976, in exchange for house or flat in Rio de Janeiro. Four bedrooms and garden (Dr C. J. Sanderson, 26 Olive Road, Cricklewood, London NW2 6TX, UK; (01) 452 8364).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

November 12, **Life-games with glass beads and molecules**, Cambridge (The Secretary, Department of Chemistry, Lensfield Road, Cambridge, UK).

November 21, **The scientist as a leader**, London (The Industrial Society, PO Box 1BQ, Robert Hyde House, 48 Bryanston Square, London W1H 1BQ).

December 2-3, **Grass weeds**, Paris (European Weed Research Society, c/o ARC Weed Research Organisation, Begbroke Hill, Yarnton, Oxford OX5 1PF, UK).

Reports and publications

Great Britain

- The Soil Association Quarterly Review*, Vol. 1, No. 1, September 1975. Editor: John Seymour. Published by The Soil Association, Walnut Tree Manor, Haughley, Stowmarket, Suffolk, IP14 3RS. Pp. 1-16. [19]
- Forestry Commission. Booklet 41. Fertilisers in the Establishment of Conifers in Wales and Southern England. By J. E. Everard. Pp. 49. (London: HMSO, 1974.) [19]
- Forestry Commission. Booklet No. 42. Field Recognition of British Elms. By J. Jobling and A. F. Mitchell. Pp. 24. (London: HMSO, 1974.) 85p net. [19]
- Forestry Commission Bulletin No. 48. Weeding in the Forest: A Work study approach. By W. O. Wittering. (London: HMSO, 1974.) £2.10. [19]
- Forestry Commission Bulletin No. 49. The Potential of Western Hemlock, Western Red Cedar, Grand Fir and Noble Fir in Britain. By J. R. Aldhouse and A. J. Low. (London: HMSO, 1974.) £1.50. [19]
- Progress in Food and Nutrition Science*, Vol. 1, No. 1, 5th May 1975. Editor-in-Chief: H. M. Sinclair. Pp. 1-64. Published as 12 issues per year. Subscription: \$80.00 per annum, postage included. (Oxford and New York: Pergamon, 1975.) [19]
- Bulletin of the British Museum (Natural History), Zoology Vol. 25 1973 index. Pp. 393-401. (London: The British Museum (Natural History).) [19]
- British Antarctic Survey. Scientific Reports No. 85. Crustacea Amphipoda from Graham Land and the Scotia Arc, Collected by Operation Tabarin and the Falkland Islands Dependencies Survey, 1944-59. By Michael H. Thurston. Pp. 89. (London: British Antarctic Survey, Natural Environment Research Council, 1974.) £4.45. [19]
- British Antarctic Survey. Scientific Reports No. 86. The Geology of the South Orkney Islands: III. Coronation Island. By Janet W. Thomson. Pp. 39 + 6 plates. (London: British Antarctic Survey, Natural Environment Research Council, 1974.) £2.60. [19]
- Bibliothèque de Travail. Les Ponts de Paris. Adapted by Eileen Daffern, assisted by Mollie Kaye and Jean Poncet. Pp. 32. (Glasgow and London: Blackie and Son Ltd., 1974.) 70p. [29]
- Bibliothèque de Travail. La Camargue. Adapted by Eileen Daffern. Assisted by Mollie Kaye and Jean Poncet. Pp. 31. (Glasgow and London: Blackie and Son Ltd., 1974.) 70p. [29]
- Flora of Tropical East Africa. Edited by R. M. Polhill. Passifloraceae. By W. J. J. O. de Wilde. Pp. 70. (London: Crown agents for Oversea Governments and Administrations, 1975. Published on behalf of the East African Community.) 80p. [59]
- The Wellcome Trust, 1972-74. Tenth Report. Pp. 124. (London: The Wellcome Trust, 1975.) [89]
- UCCA Statistical Supplement to the Twelfth Report, 1973-4. Pp. 28. (Cheltenham, Glos.: The Universities Central Council on Admissions, 1975.) £1.25. [89]
- House of Commons Paper No. 487. First Report from the Select Committee on Science and Technology: Energy Conservation. Session 1974-75. Pp. 75. (London: HMSO, 1975) 95p. [109]
- House of Commons Paper No. 504. Second Report from the Select Committee on Science and Technology: Scientific Research in British Universities. Session 1974-75. Pp. 43. (London: HMSO, 1975.) 65p. [109]
- Social Science Research Council. Research into the Social Impact of North Sea Oil Developments in Scotland: Report of an SSR Advisory Group. Pp. 52 + 13. (London: Social Science Research Council, 1975.) 50p. [109]
- British Antarctic Survey. Scientific Reports No. 78. The Geology of the South Shetland Islands: V. Volcanic Evolution of Deception Island. By P. E. Baker, I. McReath, M. R. Harvey, M. J. Roobol and T. G. Davies. Pp. 81 + 13 plates. (Cambridge: The British Antarctic Survey, Natural Environment Research Council, 1975.) £5.75. [109]
- Proceedings of the Royal Irish Academy. Vol. 75, Section B, No. 16: Upper Devonian—Lower Carboniferous Stratigraphy Along the South Coast of Dunmanus Bay, Co. Cork. By D. Naylor. Pp. 317-337 + plate 6. 50p. No. 17: Substitution Reactions of Square-Planar Platinum (II) Complexes with Bidentate Nucleophiles. By M. J. Hynes and Shiela P. Russell. Pp. 339-344. 20p. No. 18: Zoogeographical and Ecological Determinants of Collembolan Distribution. By R. E. Blackith and R. M. Blackith. Pp. 345-368. 50p. No. 19: Structure of the Gneiss Complex of Inishtrahull, Co. Donegal. By D. R. Bowes and A. M. Hopgood. Pp. 369-399 + plates 7-10. £1.15. No. 20: The Effect of Temperature on Size and Structure—II. The Body Musculature of *Cyclops agilis* (Koeck, Sars.) By E. Campbell and J. N. R. Grainger. Pp. 391-369. Vol. 75, Section B, No. 12: On Groups with Exponent Four. By S. Tobin. Pp. 115-120. 24p. (Dublin: Royal Irish Academy, 1975.) [159]
- The Nuffield Foundation. Report for the Year 1975. Pp. 127. (London: The Nuffield Foundation, 1975.) [166]
- The British Glass Industry Research Association. Annual Report 1975. Pp. 40. (Sheffield: The British Glass Industry Research Association, 1975.) [159]
- The Natural Environment Research Council. Publications Series B. No. 12: Growth Areas in the Geological Sciences. (Report of the NERC *ad hoc* Working Party on Geological Sciences for 1974.) Pp. 8. No. 11: Research in Aquatic Microbiology. (Report of the NERC Working Party on Aquatic Microbiology.) Pp. 30. (London: Natural Environment Research Council, 1975.) [189]
- British Antarctic Survey. Scientific Reports. No. 80: Ammonite Faunas of the Lower Cretaceous of South-Eastern Alexander Island. By M. R. A. Thomson. Pp. 44 + 5 plates. £2.80 net. No. 84: The Geology of the Danco Coast Graham Land. By Susan M. West. Pp. 58 + 6 plates. £4.50 net. No. 88: A Simple Empirical Method for Estimating the Height and Semi-Thickness of the F2-Layer at the Argentine Islands, Graham Land. By J. R. Dudeney. Pp. 46. £2.90 net. No. 89: The Macroliths of South Georgia. By D. C. Lindsay. Pp. 91 + 3 plates. £4.75 net. (London: British Antarctic Survey, Natural Environment Research Council, 1974.) [159]
- Imperial College of Science and Technology. Calendar 1975/76. Pp. 607. (London: Imperial College, University of London, 1975.) [169]
- Science Research Council. Academic-Industrial Collaboration in Engineering Research—a Report to the Engineering Board. Pp. 23. (London: Science Research Council, 1975.) [169]
- Bulletin of the British Museum (Natural History), Geology. Vol. 26, No. 5: Ostracods from Callovian to Tithonian Sediments of Tanzania, East Africa. By R. H. Bate. Pp. 161-223 + 14 plates. £5.15. Vol. 26, No. 2: Lower Cretaceous Ammonites from North-East England—The Hauterivian Heteromorph *Aegoceras*. By P. F. Rawson. Pp. 129-159 + 6 plates. £2.95. Historical Series. Vol. 4, No. 6: The Non-Descriptive Palaeontology of the Sowerbys' *Mineral Conchology*. By G. F. Elliott. Pp. 387-399 + 1 plate. £1. Zoology. Vol. 28, No. 5: Miscellaneous. Pp. 153-247 + 9 plates. £6.20. (London: British Museum (Natural History), 1975.) [179]
- International Union of Laboratory Animals. Third edition. Pp. 93. (Carshalton, Surrey: Medical Research Council, Laboratory Animals Centre, 1975.) [199]
- Report of the British Museum (Natural History), 1972/1974. Pp. vii + 173. 1975. £2.50 net. [199]
- Department of Industry. The Future of Real Time Technology—a Report to the Computers, Systems and Electronics Requirements Board. Pp. 69. (London: Department of Industry, 1975.) [199]
- Ordnance Survey. Annual Report, 1974/1975. Pp. 23 + 6 plates. (London: HMSO, 1975.) [199]
- Friends of the Lake District. Report and News Letters October 1975. Pp. 32. (Kendal: Friends of the Lake District, 1975.) [229]
- Freshwater Biological Association. Forty-Third Annual Report for the year ended 31 March 1975. Pp. 194. (Ambleside, Cumbria: Freshwater Biological Association, 1975.) £1. [229]
- National Institute of Agricultural Botany. Fifty-fifth Report and Accounts, 1974. Pp. 81. (Cambridge: National Institute of Agricultural Botany, 1975.) [229]
- The Open University Brain Research Group 1969-1975—a Brief Account. Pp. 30. (Milton Keynes: The Open University, Walton Hall, 1975.) [239]
- The Health Care Dilemma or "Am I Kranken, Doctor?" Pp. 24. (London: Office of Health Economics, 162 Regent Street, 1975.) [249]
- Report of the Social Science Research Council, April 1974-March 1975. Pp. 75. (London: HMCO, 1975.) £1 net. [249]
- Procter and Gamble, Ltd. Annual Report for the year ended 30th June 1975. Pp. 8. (Newcastle upon Tyne: Procter and Gamble, Ltd., 1975.) [249]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 279, No. 1290: The Intrinsic Properties of Rank and Nullity if the Lagrange Bracket in the One Dimensional Calculus of Variations. By G. R. Allcock. Pp. 487-545. UK £2.50; Overseas £2.60. Vol. 279, No. 1291: The Recovery of Structure from Fragmentary Information. By D. G. Kendall. (Review Lecture.) Pp. 547-582. UK £1.65; Overseas £1.70. Vol. 279, No. 1292: The Influence of Rotation on the Free Oscillations of the Earth. By F. A. Dahlen and M. L. Smith. Pp. 583-629. UK £1.80; Overseas £1.85. (London: The Royal Society, 1975.) [259]
- Mineral Resources Consultative Committee. Mineral Dossier No. 12: Slate. Compiled by R. N. Crockett. Pp. v + 26. 85p net. Mineral Dossier No. 13: Gypsum and Anhydrite. Compiled by A. J. G. Northolt and D. E. Highley. Pp. v + 38. £1 net. (London: HMSO, 1975.) [250]
- Council for National Academic Awards Annual Report, 1973/1974. Pp. 54. London: CNA, 344-354 Grays Inn Road, WC1, 1975.) [259]
- Mining Research and Development Establishment Annual Report, 1974/1975. Pp. 22. (London: National Coal Board, 1975.) [299]
- The Malaysian Rubber Producers' Research Association. Thirty-seventh Annual Report, July 1975. Pp. 44. (Brickendonbury, Hertford: The Malaysian Rubber Producers' Research Association, 1975.) [299]
- National Radiological Protection Board. NRPB-R19. The Identification of an Homogenous Critical Group Using Statistical Extreme-Value Theory—Application to Laverbread Consumers and the Wind-salt Effluent Discharges. By S. Beach. Pp. 14. (Harwell, Didcot: NRPB, 1974.) [299]

Other countries

- World Health Organisation. Technical Report Series, No. 571: Early Detection of Health Impairment in Occupational Exposure to Health Hazards—Report of a WHO Study Group. Pp. 80. (Geneva: WHO; London: HMSO, 1975.) Sw. Fr. 8. [19]
- Rapports des Groupes de Réflexion et de Prospective sur les Recherches Liées aux Problèmes de L'Énergie. Pp. 269. (Paris: Centre National de la Recherche Scientifique, 1975.) [19]
- Israel Environmental Protection Service. Selected Papers on the Environment in Israel No. 3. (Jerusalem: Environmental Protection Service, Prime Minister's Office, 1975.) [29]
- World Health Organisation. Selected Bibliography on Detection of Dependence-Producing Drugs in Body Fluids. Compiled by T. L. Chruseiel and M. Chruseiel. Pp. vi + 67. (Geneva: World Health Organisation, 1975.) Sw. Fr. 15. [39]
- Transactions of the American Philosophical Society. New Series Vol. 65, Part 4, 1975: The Flight of Birds: The Significant Dimensions, their Departure from the Requirements for Dimensional Similarity and the Effect on Flight Aerodynamics of that Departure. By Crawford H. Greenewalt. Pp. 67. (Philadelphia: The American Philosophical Society, July 1975.) \$7.00. [49]
- UNESCO technical papers in marine science, 20. Ichthyoplankton: Report of the CICAR Ichthyoplankton Workshop, Mexico City, 17-26 July 1974. Pp. 46. (Paris: UNESCO, 1975.) [49]
- Population Bulletin, Vol. 30, No. 3. The Elderly in America. By Leon Bouvier, Elinore Atlee and Frank McVeigh. Pp. 36. (Washington, D.C.: Population Reference Bureau, 1755 Massachusetts Avenue, 1975.) [59]
- National Institutes of Health Annual Report of International Activities, Fiscal Year 1974. Prepared by John E. Fogarty. Pp. vi + 115. (Bethesda, Maryland: United States Department of Health, Education and Welfare, 1975.) [89]
- Psycho-Physical Theory (Improved). By J. H. Greidanus. Koninklijke Nederlands Akademie van Wetenschappen. Reprinted from Proceedings, Series B. Vol. 78. (1975.) Pp. 37. (Amsterdam and Oxford, North Holland Publishing Company, 1975.) [89]
- OECD Nuclear Energy Agency. Third Activity Report, 1974. Pp. 75. (Paris: OECD Nuclear Energy Agency, 1975.) [89]
- Department of Defence, Australian Defence Scientific Service, Materials Research Laboratories, Maribyrnong, Victoria. Report 615: Underwater Marine Coatings, Elimination Reactions of Tributyltin W-Chloroalkanoates. By G. Bocksteiner, G. Glew and A. T. Phillip. Pp. 9 + 12 figures. (Melbourne: Australian Defence Scientific Service, 1974.) [89]
- Bulletin of the American Museum of Natural History, Vol. 155, Article 5. A Phylogeny and Classification of the Higher Categories of Turtles. By Eugene S. Gaffney. Pp. 387-436. (New York: American Museum of Natural History, 1975.) \$2.40. [99]
- Annals of the Transvaal Museum: Vol. 29, No. 12. The Burrowing Geckos of Southern Africa. I. (Reptilia: Gekkonidae). By W. D. Haacke. (Pretoria: Transvaal Museum, 1975.) [109]
- Alternatives: a Journal of World Policy*, Vol. 1, No. 1, March 1975. General Editor: Rajni Kothari. Pp. 1-158. Published quarterly. (Amsterdam: North-Holland Publishing Company, 1975.) [159]
- Manual on Radiation Protection in Hospitals and General Practice. Vol. 2: Unsealed Sources. By D. Frost and H. Jammet. Pp. 113. (Geneva: World Health Organisation; London: HMSO, 1975.) Sw. Fr. 24. [159]
- New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March 1975. Pp. 76. 55 cents. Report of the National Research Advisory Council for the year ended 31 March 1975. Pp. 24. 20 cents. (Wellington: Government Printer, 1975.) [159]
- Koninklijk Nederlands Meteorologisch Instituut. Mededelingen en Verhandelingen, No. 97: An Approach to the Possibilities of Forecasting Downy Mildew Infection in Onion Crops. By G. A. de Wille. Pp. 83. (De Bilt, Netherlands: Kon. Nederlands Meteorologisch Instituut, 1975.) Dfl. 17.50. [159]
- National Research Council, Canada. NRCC No. 14096: Photochemical Air Pollution—Formation, Transport and Effects. (NRC Associate Committee on Scientific Criteria for Environmental Quality.) Pp. 224. (Ottawa: Publication, NRCC/CNRC, 1975.) \$2.50. [159]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-33: Geology of the Tuadook Lake Map-Area, New Brunswick (21 J/15). By R. Skinner. Pp. 9. \$2.40. Paper 75-4: Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, April 1974, to March 1975. Pp. 29. \$2.40. Paper 75-23: Mollusca of the Strait of Canso Area. By Frances J. E. Wagner. Pp. 29 (7 plates). \$3. Paper 75-29: Experimental Hydrogeochemical Surveys of the High Lake and Hackett River Areas, Northwest Territories. By E. M. Cameron and S. B. Ballantyne. Pp. 17. \$2.40. Paper 75-37: National Report for Canada on Volcanology. Compiled by W. R. A. Baragar. Pp. 12. \$1.80. (Ottawa: Information Canada, 1975.) [159]
- United States Department of the Interior: Geological Survey. Professional Paper No. 884: Carboniferous Biostratigraphy, Northeastern Brooks Range, Arctic Alaska. By Augustus K. Armstrong and Bernard L. Mamet. Pp. iii + 29. (Washington, DC: Government Printing Office, 1975.) [159]
- Unesco. Unesco Technical Papers in Marine Science No. 21: An Intercomparison of Open Sea Tidal Pressure Sensors. (Report of SCOR working group 27: "Tides of the Open Sea".) Pp. 67. (Paris: Unesco, 1975.) [189]